EMBO
Workshop

C. elegans development, cell biology and gene expression

13 – 17 June 2018 | Barcelona, Spain

Abstract Book

meetings.embo.org/event/18-c-elegans

EMBO excellence in life sciences
CRG Centre for Genomic Regulation
The Company of Biologists
EMBO Young Investigator
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Logo design: Ahna Skop

T-shirts, mugs, bags, and other items with the Barcelona *C. elegans* meeting logo can be ordered online:

https://www.cafepress.com/celegans/15377503
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Program & Abstract Book

EMBO workshop *C. elegans* Development, Cell Biology & Gene Expression

Combined *C. elegans* Topic Meeting and European *C. elegans* meeting

June 13 - 17, 2018

Meeting Organizers

- Sander van den Heuvel. Utrecht University, NL
- Sophie Jarriault. IGBMC, FR
- Alex Hajnal. University of Zurich, CH

Co-Organizers

- Julian Ceron. Bellvitge Biomedical Research Institute - IDIBELL, ES
- Luisa Cochella. Research Institute of Molecular Pathology (IMP), AT
- Ben Lehner. Centre for Genomic Regulation, ES

Scientific Committee

- Erik Anderson, Northwersten University, USA
- Michael Barkoulas, Imperial College London, UK
- Henrik Bringmann, Max Planck Institute for Biophysical Chemistry, DE
- Olivia Casanueva, Babraham Institute, UK
- Julian Ceron, Bellvitge Biomedical Research Institute-IDIBELL, ES
- Barbara Conradt, Ludwig-Maximilians-Universität München, DE
- Thorsten Hoppe, University of Cologne, DE
- Jane Hubbard, Skirball Institute of Biomolecular Medicine, USA
- Janine Kirstein, FMP Berlin, DE
- Ben Lehner, Centre for Genomic Regulation, ES
- Christian Pohl, Goethe-Universität Frankfurt, DE
- Benjamin Podbilewicz, Technion Israel Institute of Technology, IL
- Ahna Skop, Wisconsin University, USA
- Asako Sugimoto, Tohoku University, JP
- Baris Tursun, MDC Berlin, DE

PROGRAM AT A GLANCE

	09:00 - 10:10 Auditorium **Session 2a** **Germline, Epigenetics & Sex Determination**	09:00 - 10:10 Auditorium **Session 5a** **Morphogenesis**	09:00 - 10:10 Auditorium **Session 7a** **Cell fate II (Neuronal) & Behaviour**	09:00 - 10:10 Auditorium **Session 10a** **Ageing**
	10:10 - 10:35 Coffee break	10:10 - 10:35 Coffee break	10:10 - 10:35 Coffee break	10:10 - 10:35 Coffee break
	10:35 - 11:35 Auditorium **Session 2b** **Germline, Epigenetics & Sex Determination**	10:35 - 11:35 Auditorium **Session 5b** **Morphogenesis**	10:35 - 11:40 Auditorium **Session 7b** **Cell fate II (Neuronal) & Behaviour**	10:35 - 11:35 Auditorium **Session 10b** **Ageing**
	11:35 - 13:00 Lunch	11:35 - 13:00 Lunch	11:40 - 13:00 Lunch	11:35 - 11:45 Auditorium **Closing**
	13:00 - 14:10 Auditorium **Session 3a** **Cell Biology & Early Development**	13:00 - 14:10 Auditorium **Session 6a** **Evolution & Natural variation**	13:00 - 13:55 Auditorium **Session 8a** **Metabolism & Microbe-host interactions**	
	14:10 - 14:35 Break	14:10 - 14:35 Break	13:55 - 14:20 Break	
	14:35 - 15:40 Auditorium **Session 3b** **Cell Biology & Early Development**	14:35 - 15:30 Auditorium **Session 6b** **Evolution & Natural Variation**	14:20 - 15:20 Auditorium **Session 8b** **Metabolism & Microbe-host interactions**	
	15:40 - 16:05 Coffee break	15:30 - 18:00 Port Vell Room **Poster session II**	15:20 - 15:45 Coffee break	
			15:45 - 17:00 **Session 9** **DNA repair & Cell Death**	
17:00 - 17:10 Auditorium **Opening of the meeting**	16:05 - 17:15 Auditorium **Session 4a** **Cell Fate I (postembryonic)**			
17:10 - 18:25 Auditorium **Session 1a** **Genomics & Systems Biology**	17:15 - 17:40 Break			
	17:40 - 18:35 Auditorium **Session 4b** **Cell Fate I (postembryonic)**	18:00 - 18:30 Auditorium **Keynote address** **Worm Tales**		
18:25 - 18:55 Coffee break		18:30 - 19:30 Auditorium **Special keynote lecture**		
18:55 - 20:00 Auditorium **Session 1b** **Genomics & Systems Biology**	18:35 - 21:05 Port Vell Room **Poster session I**			
20:00 - 21:00 Welcome Mixer			20:00 Conference Dinner	

GENERAL INFORMATION

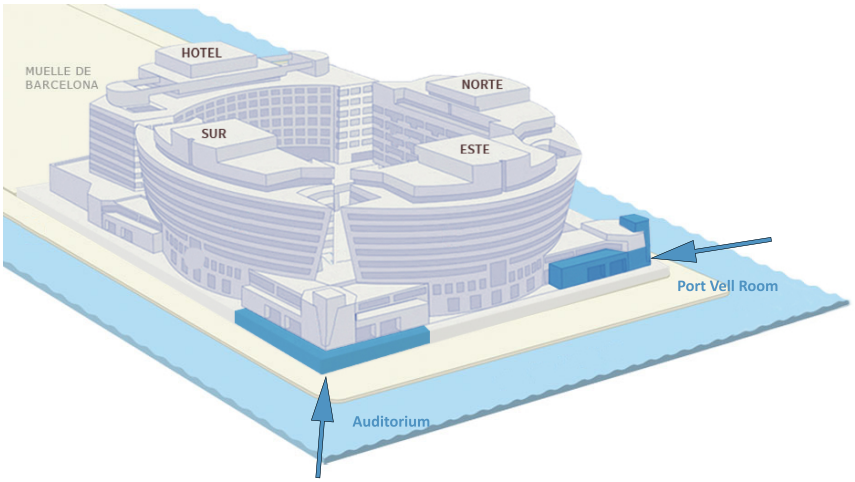
Venue

World Trade Center Barcelona

Moll de Barcelona s/n,

East building, 1st Floor,

08039, Barcelona (Spain)



Registration and Information Desk

The conference registration desk will be open according to the following schedule:

Date	Time	Location
Wednesday, June 13	13:00 – 20:00	Auditorium Hall
Thursday, June 14	08:30 – 18:00	Auditorium Hall
Friday, June 15	08:30 – 19:30	Auditorium Hall
Saturday, June 16	08:30 – 17:00	Auditorium Hall
Sunday, June 17	08:30 – 11:45	Auditorium Hall

Internet

Free WiFi is available throughout the Auditorium. User: EMBO. Password: BCN2018

Cameras, Cell Phones and Video Recording Devices

In order to protect unpublished data, the use of cameras and other recording devices in oral and poster sessions is prohibited.

Badges

Badges are required for admission to all sessions, posters, exhibit hall, and reception. If you lose your badge, a duplicate can be printed at the Conference Registration Desk for 5€.

Certificates of Attendance

All Certificates (attendance, oral talks and posters) will be sent through the meeting webpage.

Poster Competition

Poster prizes will be awarded only among postdocs and graduate students. Judges attempt to visit each poster when the authors are scheduled to present. The competition is open to postdocs and graduate student that are the first and presenting author on the poster.

Platform sessions:

All talks will be presented in the WTC auditorium.

Instructions for speakers:

Time allocation:

Speakers selected from the abstracts have been notified about the length of their talk:

15 minutes (12 min presentation and 3 min. discussion), or **5 minutes** (short, poster pitch style. Questions and discussion during the poster session).

Other oral presentations:

Special Keynote: 1 hour (50 min. presentation, 10 min discussion)

Keynote address: 30 minutes (25 min. presentation, 5 min. discussion)

Session keynotes: 35 minutes (30 min. presentation, 5 min. discussion)

Invited session speakers: 25 minutes (20 min. presentation, 5 min discussion)

Please **make sure your talk does not take more time than allotted**, as the schedule is tight.

Audio-visual

All presentations should be loaded directly onto a meeting computer at the technician cabin of the Auditorium. A Mac and PC will be available, with applications to support presentations in Powerpoint, Keynote or pdf format. In the interest of time, personal computers cannot be used for the presentations.

Presenters should bring their presentation on a USB drive and upload it well before the session in which they speak starts: morning sessions, the day before – afternoon sessions, the day before or at the end of the morning session during the lunch break. A technician will be available in the afternoon of June 13, during lunch breaks and following sessions.

Please name your presentation with the session number and your last name. The presenters should test their presentation together with the technician. Please embed movies into the presentation, but also bring them in a separate folder, to allow re-inserting and adjusting the format should problems arise.

The speakers will have a remote control with forward and reverse keys, which work like the left and right arrows on a keyboard, and can be used to start an animation/movie with an extra click.

Please arrive at the auditorium 10-15 minutes before the beginning of your session to have the chance to meet your session chair and receive some last-minute information if needed.

Poster Sessions

All posters will be presented in the *Port Vell Room*. There will be two poster sessions. The poster space will be 90 x 120 cm (width x height), which corresponds to A0 vertical format (or 16 pages in A4 format). Push pins will be available at the main entrance to the poster session.

Thursday June 14, 18.30-21.00 hr. Poster session I, **Odd-numbered** posters.

Friday June 15, 15.30-18.00 hr. Poster session II, **Even-numbered** posters

Poster numbers can be found in the abstract book and in the pdf available at the website.

All posters will be up for one day, and should be removed at the end of the poster session. After that time, posters will be removed and may be lost or discarded. The venue does not take responsibility for posters that are not removed on time.

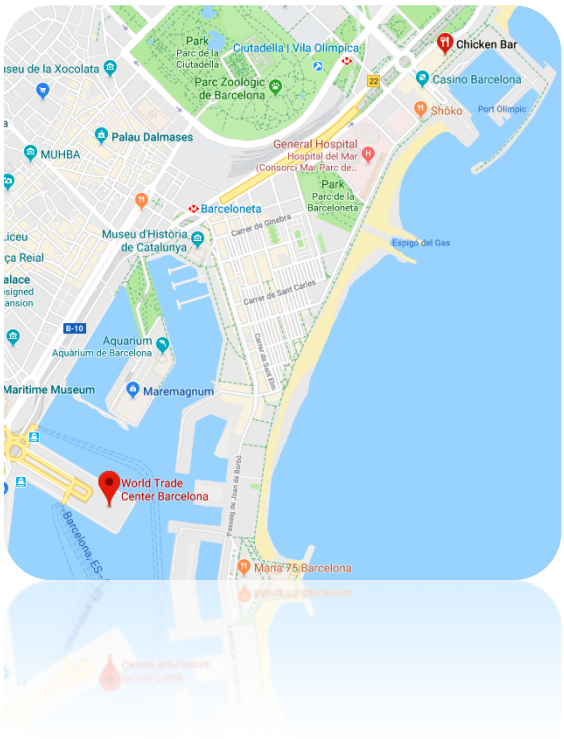
Social Events

The Welcome mixer will take place Wednesday, 13 June at 20:00 at the Hotel Eurostars Grand Marina

The Conference dinner will take place Saturday, 16 June at 20:00 at the restaurant Chicken Bar (Carrer Marina, 16 C.P. 08005 Barcelona, see map)

To register for this dinner, an email poll will be sent: please respond by indicating whether you will or will not come to the conference dinner.

Party: Dinner will be followed by a music and dance party, until 1.00 am in the same venue.



PROGRAM

Wednesday, 13 June 2018

Opening of the Meeting, General Information	
17:00-17:10	Sander van den Heuvel, Utrecht University, NL Sophie Jarriault, IGBMC, FR Alex Hajnal, University of Zurich, CH
Session 1 Genomics & Systems Biology *Chair: Ben Lehner, CRG, ES*	
17:10-17:45	**S1-01 Genome architecture and regulation** Julie Ahringer, Wellcome Trust/Cancer Research UK Gurdon Institute, UK
17:45-18:00	**S1-02 A comprehensive spatiotemporal map of embryonic gene expression** Qin Zhu, Hannah Dueck, Priya Sivaramakrishnan, Amanda Zacharias, Shaili Patel, Elicia Preston, Junhyong Kim, John Murray
18:00-18:15	**S1-03 Spatially-resolved transcriptomics in *C. elegans* identifies sex-specific differences in gene expression patterns** Annabel L. P. Ebbing, Abel Vertesy, Marco Betist, Bastiaan Spanjaard, Jan Philipp Junker, Eugene Berezikov, Alexander van Oudenaarden, Hendrik C. Korswagen
18:15-18:20	**S1-04 Quantitative RNA-seq meta analysis of alternative exon usage in C. elegans.** Denis Dupuy, Nicolas Tourasse
18:20-18:25	**S1-05 Systematic analysis of cell cycle phases reveals only a single cell pair develops with full cell cycle during *C. elegans* embryogenesis** Ming-Kin Wong, Vincy Wing Sze Ho, Xiaotai Huang, Runsheng Li, Lu-yan Chan, Hon Chun Kaoru Ng, Hong Yan, Zhongying Zhao
18:25 - 18:55	Coffee break
18:55-19:20	**S1-06 *C. elegans* locomotion** Andre Brown, Imperial College London, UK
19:20-19:35	**S1-07 Gene expression of an animal germline near single cell resolution** Asija Diag, Marcel Schilling, Filippos Klironomos, Nikolaus Rajewsky
19:35-19:50	**S1-08 Application of Imaging mass spectrometry for *C. elegans*** Yoshishige Kimura, Tomomi Kaneko, Satoka Aoyagi
19:50-19:55	**S1-09 Adapting a proximity labeling technique to identify novel non-centrosomal MTOC proteins in *C. elegans*** Ariana D. Sanchez, Tess Branon, Alice Y. Ting, Jessica L. Feldman
19:55-20.00	**S1-10 The THAP domain protein LIN-36 functions with the DREAM complex to repress transcription of proliferation promoting genes during quiescence** Csenge Gal, Alex Appert, Chiara Cerrato, Ni Huang, Julie Ahringer
20:00	Welcome Mixer

Thursday, 14 June 2018	
Session 2: Germline, Epigenetics & Sex Determination *Chair: Richard Roy, Department of Biology, DBRI, McGill University, Canada*	
9:00-9:35	**S2-01 Counting to One: Patterning of Meiotic Recombination in *C. elegans*** Abby Dernburg, UC Berkeley, USA
9:35-9:50	**S2-02 Tissue- and sex-specific small RNAomes reveal sex differences in response to the environment** Alexandra Bezler, Fabian Braukmann, Sean West, Fabio Piano, Kristin Gunsalus, Eric Miska, Laurent Keller, et al.
9:50-10:05	**S2-03 Functional characterisation of RBP-9, an RNA Pol II subunit that is required for piRNA-mediated gene silencing** Lisa Lampersberger, Ahmet Can Berkyurek, Eva Maria Weick, Isabela Navarro, Kin Man Suen, Alper Akay, David Jordan, Eric Miska
10:05-10:10	**S2-04 Identification and characterization of a fertilization signal pathway for female meiosis in *C. elegans*.** Rudra Banerjee, Jens Herzog, Martin Srayko
10:10 - 10:35	Coffee break
10:35-11:00	**S2-05 Determinants of cell fate stability in nematodes** Peter Meister, Institute of Cell Biology, University of Bern, CH
11:00-11:15	**S2-06 Towards identifying genes regulating multipotency and differentiation in the SGP/hmc cell fate decision** Laura Mathies, Andrew Davies, Jill Bettinger
11:15-11:30	**S2-07 SPR-5; MET-2 maternal reprogramming antagonizes H3K36me3 in the transgenerational control of germline versus soma** Brandon Carpenter, David Katz
11:30-11:35	**S2-08 AMPK regulates germline stem cell quiescence and integrity through effects on a small RNA pathway** Pratik Kadekar, Richard Roy
11:35 - 13:00	Lunch
Session 3: Cell biology & Early Development *Chair: Christian Pohl, Uni Köln (DE)*	
13:00-13:35	**S3-01 Novel facets of cell polarity in the *C. elegans* zygote** Pierre Gönczy, Swiss Federal Institute of Technology Lausanne (EPFL), CH
13:35-13:50	**S3-02 A two-step mechanism for the inactivation of MTOC function at the centrosome** Jeremy Magescas, Jennifer C. Zonka, Jessica L. Feldman
13:50-14:05	**S3-03 Aurora-A triggers local and global inhibition of contractile actomyosin networks independently of its role in centrosome maturation** Peng Zhao, Masatoshi Nishikawa, Fumio Motegi
14:05-14:10	**S3-04 Optogenetic control of spindle positioning** Ruben Schmidt, Lars-Eric Fielmich, Anna Akhmanova, Sander van den Heuvel
14:10 - 14:35	Break
14:35-15:00	**S3-05 Why parthenogenetic females of Mesorhabditis belari would sire males whose DNA is not transmitted to females?** Marie Delattre, Université de Lyon (ENS), FR

15:00-15:15	**S3-06 Patterns of contact cue diversify cell division orientation with myosin flow** Kenji Sugioka, Bruce Bowerman
15:15-15:30	**S3-07 Embryonic development in *C. elegans*: questioning the importance of yolk** Ellen Geens, Pieter Van de Walle, Liliane Schoofs, Liesbet Temmerman
15:30-15:35	**S3-08 Channel Nucleoporins recruit the Polo-like kinase PLK-1 to the Nuclear Pore Complexes in prophase to direct Nuclear Envelope Breakdown in C. elegans embryos** Lionel Pintard, Lisa Martino, Stéphanie Morchoisne-Bolhy, Dhanya Cheerambathur, Lucie Van Hove, Julien Dumont, Nicolas Joly, Arshad Desai, Valerie Doye
15:35-15:40	**S3-09 Towards understanding the importance of the unequal first cleavage of *C. elegans* embryos** Radek Jankele, Rob Jelier, Pierre Gönczy
15:40 - 16:05	Coffee break
Session 4: Cell Fate I (postembryonic) *Chair: Michael Barkoulas, Imperial College London, UK*	
16:05-16:40	**S4-01 Orienting cell polarity by Wnt signaling** Hitoshi Sawa, National Institute of Genetics, JP
16:40-16:55	**S4-02 In vivo reprogramming of coelomocytes** Anna Reid, Ismail Özcan, Margaux Quiniou, Andreas Ofenbauer, Baris Tursun
16:55-17:10	**S4-03 A natural transdifferentiation event involving mitosis is empowered by integrating signaling inputs with conserved plasticity factors** Claudia Riva, Christelle Gally, Martina Hadjuskova, Sophie Jarriault
17:10-17:15	**S4-04 Cross-talk between the NOTCH and hypoxia response pathways modulates RAS/MAPK-mediated cell fate decisions in *C. elegans*** Sabrina Maxeiner, Judith Grolleman, Tobias Schmid, Jan Kammenga, Alex Hajnal
17:15 - 17:40	Break
17:40-18:05	***EMBO Young Investigator Lecture*** **S4-05 Roles of microRNAs in embryonic development** Luisa Cochella, Research Institute of Molecular Pathology (IMP), AT
18:05-18:20	**S4-06 Partial versus complete loss of SWI/SNF function leads to opposite cell division phenotypes** Molly Godfrey, Aniek van der Vaart, Vincent Portegijs, Sander van den Heuvel
18:20-18:35	**S4-07 The evolutionarily conserved DEAD-box helicase DDX-23 functions in stem cell biology** Akiko Doi, Bob Horvitz
Poster session	
18:35 - 21:05	Poster session I with snacks and drinks

Friday, 15 June 2018

Session 5: Morphogenesis

Chair: Benjamin Podbilewicz, Technion Israel Institute of Technology, IL

9:00-9:35	**S5-01 The Basement Membrane Toolkit: Looking Outside the Cell** David Sherwood, Duke University, USA
9:35-9:50	**S5-02 The Pre-Replication Complex Governs the Invasive Cell Fate of the Caenorhabditis Elegans Anchor Cell in a Replication-Independent Manner** Evelyn Lattmann, Ting Deng, Vibhu Prasad, Charlotte Lambert, Michael Daube, Ossia Eichhoff, Urs Greber, Reinhard Dummer, Mitch Levesque, Alex Hajnal, et al.
9:50-10:05	**S5-03 Extrinsic stress triggers actin-based viscoplasticity to drive progressive body axis elongation** Alicia Lardennois, Gabriella Pásti, Teresa Ferraro, Julien Pontabry, David Rodriguez, Flora Llense, Samantha Kim, Christelle Gally, Michel Labouesse
10:05-10:10	**S5-04 Contractile ring-dependent cytoplasmic partitioning in non-mitotic germ cells** Chelsea Maniscalco, Jeremy Nance
10:10 - 10:35	Coffee break
10:35-11:00	**S5-05 Building and shaping seamless tubes: repurposing a cell-cell fusogen for membrane trafficking** Meera Sundaram, University of Pennsylvania, USA
11:00-11:15	**S5-06 Activation of Aurora A kinase by TPXL-1 clears contractile ring proteins from the cell poles during cytokinesis** Sriyash Mangal, Jennifer Sacher, Taekyung Kim, Daniel Sampaio Osório, Fumio Motegi, Ana Carvalho, Karen Oegema, Esther Zanin
11:15-11:30	**S5-07 Microtubule Dynamics Scale with Cell Size to Set Spindle Length and Assembly Timing** Benjamin Lacroix, Gaëlle Letort, Laras Pitayu, Jeremy Sallé, Julie Canman, Nicolas Minc, François Nedelec, Julien Dumont
11:30-11:35	**S5-08 *C. elegans* blastomeres clear the corpse of the second polar body by LC3-associated phagocytosis** Gholamreza Fazeli, Maurice Stetter, Jaime Lisack, Ann Wehman
11:35 - 13:00	Lunch

Session 6: Evolution & Natural Variation

Chair: Erik Anderson, Northwersten University, USA

13:00-13:35	**S6-01 Wild *C. elegans*** Marie-Anne Félix, IBENS, FR
13:35-13:50	**S6-02 Small peptide mediated self-recognition prevents cannibalism in predatory nematodes** James Lightfoot, Martin Wilecki, Christian Roedelsperger, Eduardo Moreno, Ralf Sommer
13:50-14:05	**S6-03 Dissecting the sources of phenotypic variation among genetically identical individuals growing in the same environment** Mirko Francesconi, Marcos Perez, Ben Lehner
14:05-14:10	**S6-04 Molecular identification of vulval developmental defects in the non-model nematode Oscheius tipulae via mapping-by-sequencing** Amhed Missael Vargas Velazquez, Fabrice Besnard, Clement Dubois, Marie-Anne Felix
14:10 - 14:35	Break

14:35-15:00	**S6-05 There is more to genetic variation than you think: from gene mapping to complex perturbation analyses** Jan Kammenga, Wageningen University & Research, NL
15:00-15:15	**S6-06 Genome sequence and evolution of the asexual nematode Diploscapter pachys** Kristin Gunsalus, Helene Fradin, Karin Kiontke, Ryan Baugh, Fabio Piano, David Fitch, et al.
15:15-15:20	**S6-07 Single-Molecule Real Time sequencing reveals large structural variants in natural isolates of *C. elegans*** Cristian Riccio, Martin Hemberg, Eric Miska
Poster session	
15:30 - 18:00	Poster session II with snacks and drinks
Keynote address *Introduced by: Ahna Skop, UW-Madison, USA*	
18:00 - 18:30	**Worm Tales** John White, Emeritus Professor, Laboratory of Cell & Molecular Biology, University of Wisconsin, Madison, Wisconsin, US
Special keynote lecture *Introduced by: David Sherwood, Duke University, USA*	
18:30 - 19:30	**Comprehensively understanding *C. elegans* part 17: Dauer Development** Paul Sternberg, Caltech, USA

Saturday, 16 June 2018

Session 7: Cell fate II (Neuronal) & Behaviour *Chair: Henrik Bringmann, Max Planck Institute for Biophysical Chemistry, DE*	
9:00-9:35	**S7-01 Homeoboxes build the *C. elegans* nervous system** Oliver Hobert, Howard Hughes Medical Institute, USA
9:35-9:50	**S7-02 Glia and pioneer neurons direct hierarchical circuit formation through non-canonical redundant pathways of axon guidance in *C. elegans*** Georgia Rapti, Shai Shaham
9:50-10:05	**S7-03 Repurposing of the kinetochore machinery during neuronal development** Dhanya Cheerambathur, Bram Prevo, Arshad Desai
10:05-10:10	**S7-04 Dissecting the contribution of microRNAs to nervous system development and function** Chiara Alberti, Jingkui Wang, Luisa Cochella
10:10 - 10:35	Coffee break
10:35-11:00	**S7-05 A direct glia-to-neuron cell fate switch ensures nimble manoeuvres during male mating** Arantza Barrios, UCL, UK
11:00-11:15	**S7-06 Regulation of Long-Term Behavioral Patterns and Individuality across Development** Shay Stern, Christoph Kirst, Cornelia I. Bargmann

11:15-11:30	**S7-07 trp-1 and trp-2 TRPC channels mediate proprioceptive regulation of *C. elegans* locomotion** Jihye Yeon, Jinmahn Kim, Doyoung Kim, Hyunmin Kim, Daewon Moon, Kyuhyung Kim
11:30-11:35	**S7-08 Embryonic Exposure to Amphetamine Reduces Gene Expression of the Dopamine Transporter** Lucia Carvelli, Ganesh Ambigapathy, Talus McCowan, Archana Dhasarathy
11:35-11:40	**S7-09 Sexy learning in *C. elegans*: Integration of conflicting experiences** Laura Molina-Garcia, Sergio Benavides-Laconcha, Arantza Barrios
11:40 - 13:00	Lunch
Session 8: Metabolism & Microbe-host interactions *Chair: Olivia Casanueva, Brabaham Institute, UK*	
13:00-13:35	**S8-01 A persistence detector for transcriptional metabolic network rewiring** Marian Walhout, University of Massachusetts Medical School, USA
13:35-13:50	**S8-02 Neurohormonal signalling via a cytosolic sulfotransferase controls insulin sensitivity of *C. elegans*** Nick Burton, Vivek Dwivedi, Kirk Burkhart, Rebecca Kaplan, L. Ryan Baugh, H. Robert Horvitz
13:50-13:55	**S8-03 WormJam: A consensus *C. elegans* Metabolic Reconstruction and Metabolomics Community** Janna Hastings, Nicolas LeNovere, Michael Witting, Olivia Casanueva
13:55 - 14:20	Break
14:20-14:45	**S8-04 Host-Environment Interactions: Metabolic cross-talk for Ageing and Cancer** Filipe Cabreiro, University College London, UK
14:45-15:00	**S8-05 Rapid recruitment of non-centrosomal microtubules is required for immune activation after wounding** Clara Taffoni, Shizue Omi, Caroline Huber, Jolanta Polanowska, Jonathan Ewbank, Nathalie Pujol
15:00-15:15	**S8-06 Characterization of the starvation survival response mediated by the elongation factor kinase efk-1/eEF2K** Forum Bhanshali, Andy An, Jennifer Watts, Asad Jan, Poul Sorensen, Stefan Taubert
15:15-15:20	**S8-07 You are what you experience: The impact of environment on cellular identity** Sarah Becker, Marie-Charlotte Morin, Séverine Mangold, Sophie Jarriault
15:20 - 15:45	Coffee break
Session 9: DNA repair & cell death *Chair: Barbara Conradt, Ludwig-Maximilians-Universität München, DE*	
15:45-16:20	**S9-01 DNA damage responses in aging and disease: lessons from the worm** Björn Schumacher, Institute for Genome Stability in Ageing and Disease, DE
16:20-16:35	**S9-02 EFF-1 fusogen promotes phagosome sealing during cell process clearance** Piya Ghose, Alina Rashid, Peter Insley, Anupriya Singhal, Pavak Shah, Yun Lu, Zhirong Bao, Shai Shaham
16:35-16:40	**S9-03 Maintenance of genome integrity by Mi2** Carolyn Turcotte, Solomon Sloat, Julia Rigothi, Erika Rosenkranse, Alexandra Northrup, Nicolas Andrews, Paula Checchi

16:40-16:55	**S9-04 Genome-wide RNAi screen in *C. elegans* identifies compromised mitochondrial protein import as a signal for the induction of UPRmt** Stephane Rolland, Sandra Schneid, Melanie Schwarz, Elisabeth Rackles, Christian Fischer, Simon Hauessler, Saroj Regmi, Assa Yeroslaviz, Bianca Habermann, Eric Lambie, Barbara Conradt
16:55-17:00	**S9-05 The cisd gene family regulates physiological germline apoptosis through ced-13 and the canonical cell death pathway in Caenorhabditis elegans** Skylar King, Chipo Gray, Luhua Song, Rachel Nechushtai, Tina Gumienny, Ron Mittler, Pamela Padilla
20:00	Conference Dinner

Sunday, 17 June 2018

Session 10: Ageing

Chair: Thorsten Hoppe, University of Cologne, Institute for Genetics and CECAD, DE

9:00-9:35	**S10-01 Small nucleoli are a cellular hallmark of longevity** Adam Antebi, Max Planck Institute for Biology of Ageing, Cologne DE
9:35-9:50	**S10-02 Endosomal/autophagic regulation of the DAF-16 transcription factor** Icten Meras, Laëtitia Chotard, Christian E. Rocheleau
9:50-10:05	**S10-03 TORC2 regulates the maturation of endosome via SGK-1 in the intestine of *C. elegans*** Yijian Yan, Wenjing Qi, Ralf Baumeister
10:05-10:10	**S10-04 In vivo luminescent ATP *C. elegans* sensor strains for drug discovery in age-related diseases – an update.** Cristina Lagido
10:10 - 10:35	Coffee break
10:35-11:00	**S10-05 Modifiers of age-related protein aggregation and toxicity** Ellen Nollen, European Research Institute for the Biology of Ageing, DE
11:00-11:15	**S10-06 GSK-3 intestinal activity impacts mitochondrial function and ageing** Francisco Javier García-Rodríguez, Annmary Paul Erinjeri, Artur Bastos Lourenço, Mary Doherty, Phillip Whitfield, Peter Askjaer, Marta Artal-Sanz
11:15-11:30	**S10-07 UNC-120/SRF independently controls muscle aging and lifespan in Caenorhabditis elegans** Florence Solari, Adeline Mergoud dit Lamarche, Laurent Molin, Laura Pierson, Marie-Christine Mariol, Kathrin Gieseler, Jean-Louis Bessereau
11:30-11:35	**S10-08 Assessing involvement of the four C. elegans ACADSB orthologues in (healthy) lifespan and metabolism** Brecht Wouters, Clara Verschuuren, Ellen Geens, Winnok H. De Vos, Bart P. Braeckman, Ineke Dhondt, Liliane Schoofs, Liesbet Temmerman
Closing of the Meeting	
11:35 - 11:45	Sander van den Heuvel, Utrecht University, NL Sophie Jarriault, IGBMC, FR Alex Hajnal, University of Zurich, CH

POSTER LIST

Poster session I

P-01 Maternal age generates phenotypic variation among genetically identical individuals in *C. elegans*
Marcos Francisco Perez, Mirko Francesconi, Ben Lehner

P-03 Ribosomal RNA methylation by rram-1 modulates healthy lifespan and development
Markus Schosserer, Jarod A. Rollins, Clemens Heissenberger, Fabian Nagelreiter, Santina Snow, Aric Rogers, Johannes Grillari

P-05 A novel set of non-coding RNAs have critical roles in nematode spliced leader trans-splicing
Rotimi Fasimoye, Bernadette Connolly, Berndt Müller, Jonathan Pettitt

P-07 *C. elegans* Natural Diversity Resource
Robyn Tanny, Erik Andersen

P-09 Satellites in nematodes: a standing puzzle
Juan A. Subirana, M. Mar Albà, Xavier Messeguer

P-11 You are what you experience: The impact of environment on cellular identity
Sarah Becker, Marie-Charlotte Morin, Séverine Mangold, Sophie Jarriault

P-13 Sumoylation regulates protein dynamics during meiotic chromosome segregation
Ronald Hay, Federico Pelisch

P-15 Systematic analysis of atx-2 suppressors reveals a novel regulator of PAR-5/14-3-3sigma function during mitosis in Caenorhabditis elegans.
Megan Gnazzo, Alex Villarreal, Ahna Skop

P-17 Connecting social behaviour and phenotypic plasticity through the nekl-4 kinase in Pristionchus pacificus nematodes
Eduardo Moreno, Maša Lenuzzi, Christian Rödelsperger, Hanh Witte, Ralf Sommer

P-19 Post-mitotic roles of MEL-28 in gene expression and lifespan regulation
Raquel Romero Bueno, Celia María Muñoz Jiménez, Peter Askjaer, Agnieszka Dobrzynska, Georgina Gómez Saldivar

P-21 Pattern formation by dancing
Daniel Findeis, Christian Hennig, Ralf Schnabel

P-23 Mixing of parental genomes after fertilization in *C. elegans* involves a stepwise pronuclear membrane fusion process that connects the two pronuclei through multiple fenestrations
Orna Cohen-Fix, Mohammad Rahman, Adam Harned, Irene Chang, Kedar Narayan

P-25 Tissue-specific repair activity of ERCC1/XPF in *C. elegans*
Mariangela Sabatella, Karen Thijssen, Wim Vermeulen, Hannes Lans

P-27 Study of the fate of cellulose in *C. elegans*, its impacts on the nematode's metabolism and stress response
Gerrit Bredeck, Luo Zhongrui, Soledad Roig, Anna Laromaine

P-29 Epigenetic regulation of neuronal cell migration
Steffen Nørgaard, Laura Boreggio, Lisa Salcini

P-31 The role of protein sumoylation in anchor cell invasion
Aleksandra Fergin, Evelyn Lattmann, Charlotte Lambert, Alex Hajnal

P-33 Clarinet (CLA-1) and the ryanodine receptor (UNC-68) interact in Ca2+ control in nerve cells.
Célia Ferreira, Marie-Anne Shaw, Ian Hope

P-35 Identifying new functions of the lin-3 egf/ let-23 egfr pathway through tissue-specific recombination
Silvan Spiri, Louisa Mereu, Alex Hajnal

P-37 Involvement of the human lipodystrophy protein SEIPIN in Caenorhabditis elegans eggshell formation and early embryonic development
Sheng-Wen Chen, Leng-Jie Huang, Chao-Wen Wang

P-39 GCY-22 acts in salt-sensing signalling pathway at the tip of the ASER neuron cilium
Servaas van der Burght, Suzanne Rademakers, Jacque-Lynne Johnson, Michel Leroux, Gert Jansen

P-41 Pro-longevity frataxin suppression in *C. elegans* reduces tumor formation by dampening the RAS/MAPk signaling
Anjumara Shaik, Sara Maciej, Natascia Ventura

P-43 Impact of a familial hyperglycemia-associated MDH2/mdh-2 mutation on insulin/IGF-1 signaling
Luca Pannone, Prapaporn Jungtrakoon, Serena Pezzilli, Antonella Marucci, Montserrat Porta-de-la-Riva, Julian Cerón, Lorella Marselli, Vincenzo Trischitta, Alessandro Doria, Sabrina Prudente, Simone Martinelli, et al.

P-45 Assessing involvement of the four C. elegans ACADSB orthologues in (healthy) lifespan and metabolism
Brecht Wouters, Clara Verschuuren, Ellen Geens, Winnok H. De Vos, Bart P. Braeckman, Ineke Dhondt, Liliane Schoofs, Liesbet Temmerman

P-47 Establishment of signaling interactions with cellular resolution for every cell cycle of embryogenesis
Zhongying Zhao, Long Chen, Vincy Ho, Ming-Kin Wong, Xiao-tai Huang, Hong Yan

P-49 Towards a conditional bipartite gene expression system for *C. elegans*
Iskra Katic, Helge Großhans

P-51 Mitochondrial biogenesis and mitophagy are processes that are not required for lifespan extension by axenic dietary restriction in *C. elegans*.
Bart Braeckman, Lieselot Vandemeulebroucke

P-53 Intrinsic and extrinsic factors influencing the cellular response to the chemotherapeutic agent cisplatin
Carmen Martínez Fernández, Jeremy Vicencio, Francisco Javier García Rodríguez, Alberto Villanueva, Ernest Nadal, Julián Cerón

P-55 Variability in the timing of a β-catenin pulse biases a stochastic cell fate decision
Jason Kroll, Jasonas Tsiaxiras, Jeroen van Zon

P-57 The Caenorhabditis elegans Integrator Complex. Transcription beyond the snRNAs.
Eva Gómez-Orte, Beatriz Sáenz-Narciso, Angelina Zheleva, Begoña Ezcurra, María de Toro, Hilde Nilsen, María P. Sacristán, Ralf Schnabel, Juan Cabello

P-59 Conserved roles of the UFM1 cascade in *C. elegans*
Jens Daniel, Charlotte Sophia Kaiser, Adrian ter Steege, Dominique Bonneau, Eva Liebau

P-61 Regulation of Caenorhabditis elegans primordial germ cell abscission
Audrey Herrmann, Eugénie Goupil, Rana Amini, Jean-Claude Labbé

P-63 SEL-5 kinase role in anterio-posterior cell outgrowth and migration
Filip Knop, Marie Macůrková

P-65 The cisd gene family regulates physiological germline apoptosis through ced-13 and the canonical cell death pathway in Caenorhabditis elegans
Skylar King, Chipo Gray, Luhua Song, Rachel Nechushtai, Tina Gumienny, Ron Mittler, Pamela Padilla

P-67 Mutation in a novel P. aeruginosa acyl-CoA dehydrogenase gene enhances host mitochondrial UPR activity and survival during infection
Mark Pellegrino, Siraje Mahmud

P-69 Pim-related kinases selectively regulate sensory functions in *C. elegans*
Karunambigai Kalichamy, Kaisa Ikkala, Jonna Pörsti, Niina Santio, Sweta Hja, Carina Holmberg, Päivi Koskinen

P-71 Compression of the embryo is resolved by cell focussing
Christian Wartenberg, Christian Hennig, Ralf Schnabel

P-73 Using a forward genetic screen to identify suppressors of TDP-43 induced-toxicity
Mandy Koopman, Renée Seinstra, Ellen Nollen

P-75 Chronic statin exposure increases the lifespan of *C. elegans* in a DAF-16 (hFOXO3a) dependent manner
Andreas Jahn, Gerhard Fritz, Sebastian Honnen

P-77 pig-1 MELK is required for the non-random segregation of 'anti-apoptotic' potential during asymmetric NSM neuroblast division
Hai Wei, Barbara Conradt

P-79 The molecular basis of natural variation in oxidative stress response in Caenorhabditis elegans
Jana J Stastna, L Basten Snoek, Nell Nei, Yiru Wang, Joost AG Riksen, Jan E Kammenga, Simon C Harvey

P-81 Ufmylation in Caenorhabditis elegans
Charlotte Kaiser, Jens Daniel, Kevin Gilhaus, Sarah Hoedtke, Eva Liebau

P-83 Benzothiazepine CGP37157 extends lifespan in *C. elegans* worms
Paloma García-Casas, Jessica Arias-del-Val, Pilar Alvarez-Illera, Rosalba I Fonteriz, Mayte Montero, Javier Alvarez

P-85 A Novel Strategy to Control Protein Misfolding Diseases and Aging: Molecular Mechanisms of Transcellular Chaperone Signalling in *C. elegans*
Jo Miles, Patricija van Oosten-Hawle, David Westhead

P-87 H3K4 methylation regulators associated to intellectual disability control axon guidance
Benedetta Attianese, Steffen Nørgaard, Lisa Salcini

P-89 Characterization of PID-1 complex and its role in 21U-RNA formation in C.elegans
Nadezda Podvalnaya, Ricardo J. Cordeiro Rodrigues, Sabrina Dietz, Falk Butter, Antonio M. J. Domingues, René F. Ketting

P-91 Wnt ligands regulate the asymmetric divisions of neuronal precursors in the *C. elegans* embryo
Shilpa Kaur, Sabrina Murgan, Pauline Mélénec, Guillaume Bordet, Pierre Recouvreux, Pierre-Francois Lenne, Vincent Bertrand

P-93 Population density affects recovery from L1 arrest via insulin signalling
Alejandro Mata-Cabana, Mª Jesús Rodríguez-Palero, Laura Gómez-Delgado, Marta Artal-Sanz, María Olmedo

P-95 The role of Crumbs in *C. elegans* larval epithelia
Victoria G Castiglioni, Mike Boxem

P-97 Functional characterization of ikb-1 and nfki-1, homologs of a negative regulator of NF-κB pathway
David Brena, Montserrat Porta de la Riva, Eric Cornes, Joan Bertran, Lluís Campos, Dmytro Kukhtar, Albert García López, Anna Bigas, Lluís Espinosa, Julián Cerón

P-99 Using lineage-tracing to study how histone methylation regulates embryonic cell fate decisions
Juan D. Rodriguez, Brandon Carpenter, David J. Katz

P-101 Optogenetic control of spindle positioning
Ruben Schmidt, Lars-Eric Fielmich, Anna Akhmanova, Sander van den Heuvel

P-103 Dissecting centriole elimination during embryogenesis
Nils Kalbfuß, Marie Pierron, Pierre Gönczy

P-105 Modelling human cancer SF3B1 mutations in *C. elegans*
Xènia Serrat, Anna Esteve, Sol Katzman, Alan Zahler, Julián Cerón

P-107 Mapping a novel oxidative stress response pathway involving NHR-49
Kelsie Doering, Zoe DeBoer, Grace Goh, Stefan Taubert

P-109 Effects of autophagy gene downregulation on the ubiquitin-proteasome system in *C. elegans*.
Sweta Jha, Carina Holmberg-Still

P-111 The role of the RhoGEF ect-2 in RAS/MAPK-induced germ cell death
Tea Kohlbrenner, Simon Berger, Kirsti Arumäe, Tinri Aegerter-Wilmsen, Xavier Casadevall i Solvas, Andrew deMello, Alex Hajnal

P-113 Study of development into a diapause stage in *C. elegans*
Daisy S. Lim, Nari Kim, Junho Lee

P-115 The role of basolateral polarity regulators in epithelial tissue homeostasis
Amalia Riga, Helena Pires, Victoria Garcia, Mike Boxem

P-117 The role of ribosomal DNA copy number in *C. elegans* ageing
Andre Zylstra, Olivia Casanueva, Jonathan Houseley

P-119 DAF-21/Hsp90 is required for *C. elegans* longevity by ensuring DAF-16/FOXO isoform A function
Milán Somogyvári, Eszter Gecse, Csaba Sőti

P-121 Benzaldehyde-induced aversion and adaptive cellular responses in *C. elegans*
Gábor Hajdú, István Taisz, Csaba Sőti

P-123 Characterization of the Capicua homolog GEI-3 in *C. elegans*
Laura Rodríguez-Muñoz, Xènia Serrat, Julián Cerón, Gerardo Jiménez

P-125 Three pathways regulate spindle directions in three dimensions
Tania Sastradihardja, Christian Hennig, Frank Eggert, Ralf Schnabel

P-127 Investigating the conserved mechanosensory function of *C. elegans* tmc-1
Eva Kaulich, William R Schafer

P-129 Towards understanding the importance of the unequal first cleavage of *C. elegans* embryos
Radek Jankele, Rob Jelier, Pierre Gönczy

P-131 Penetrance of the Weismann barrier: An endoribonuclease in the soma protects germline immortality in *C. elegans*
Ralf Baumeister, Wolfgang Maier, Dietmar Pfeifer, Erika D von Gromoff

P-133 How does the non-canonical Wnt receptor CAM-1/Ror2 control neuroblast polarity?
Christa van der Veen, Lorenzo Rella, Hendrik Korswagen

P-135 Role and regulation of the MT-severing enzyme Katanin in *C. elegans*
Nicolas Joly, Eva Beaumale, Lucie Van Hove, Lionel Pintard

P-137 Germline-specific protein interaction network of the germline fate-protecting chromodomain protein MRG-1
Gülkiz Baytek, Alexander Gosdschan, Martina Hajduskova, Marlon Kazmierczak, Philipp Mertins, Baris Tursun

P-139 LITE-1 AS AN OPTOGENETIC TOOL - CHARACTERIZATION AND SIGNAL-PATHWAY
Marcial Alexander Engel, Franziska Hannig, Dana Maureen Hebchen, Bojana Languille, Christina Schüler, Alexander Gottschalk

P-141 Active RHO-1 forms clusters at the equatorial cortex during cytokinesis.
Jennifer Sacher, Esther Zanin

P-143 Systematic analysis of cell cycle phases reveals only a single cell pair develops with full cell cycle during *C. elegans* embryogenesis
Ming-Kin Wong, Vincy Wing Sze Ho, Xiaotai Huang, Runsheng Li, Lu-yan Chan, Hon Chun Kaoru Ng, Hong Yan, Zhongying Zhao

P-145 New roles for microtubules in zygote polarity
Jack Adam Martin, Josana Rodriguez

P-147 Generation & implementation of membrane-bound adenylyl cyclases as optogenetic tools with CNG-gated ion channels in Caenorhabditis elegans
Thilo Henß, Jatin Nagpal, Shiqiang Gao, Ulrike Scheib, Alexander Hirschhäuser, Franziska Schneider-Warme, Peter Hegemann, Georg Nagel, Alexander Gottschalk

P-149 Investigating in vivo variation in the strength of the spindle assembly checkpoint
Abigail Gerhold, Vincent Poupart, Paul Maddox, Jean-Claude Labbé

P-151 A novel tumor related gene, HPO-11/NRBP affects mRNA fate in *C. elegans*
Qian Zhao, Wenjing Qi, Ralf Baumeister

P-153 Heparan sulfate proteoglycans roles in morphogenesis and nervous system development in *C. elegans*
Marianne Moore, Lise Rivollet, Dan Shaye, Claire Bénard

P-155 Exploring the functional conservation of a deeply conserved animal microRNA
Paula Gutiérrez Pérez, Anna Schrempf, Luisa Cochella

P-157 Oligomerization of the RZZ (Rod-Zwilch-Zw10) complex drives expansion of the kinetochore corona

Cláudia Pereira, Rita Reis, José Gama, Dhanya Cheerambathur, Ana Carvalho, Reto Gassmann

P-159 Molecular mechanisms of synaptic tiling

Kota Mizumoto

P-161 Developmental regulation of germline syncytium organization in *C. elegans*

Jack Bauer, Mei Zhen, Jean-Claude Labbé

P-163 COPAS Vision is new instrumentation that can take pictures and sort worms

Rock Pulak, Julia Thompson, Tom Mullins, Mariya Lomakina, Bruce Holcombe, Mikalai Malinouski, Chris Bogan

P-165 Exploring the role of the ULP-2 SUMO protease in the germline

Ulrike Bening, Marana Abboud, Limor Broday

P-167 Suppressor analysis reveals new genes involved in embryonic cell migration

Vida Praitis, Zoe Scott-Nevros, Haonan Sun, Shaina Zarkin-Scott

P-169 Analysis of the transcriptional basis of natural transdifferentiation in *C. elegans*

Jaime Osuna Luque, Peter Meister, Sophie Jarriault

P-171 A genetic screen for morphogenesis-defective, temperature-sensitive mutants in Caenorhabditis elegans

Molly Jud, Josh Lowry, Thalia Padilla, Erin Clifford, Yuqi Yang, Alexander Miller, Hong Shao, Nhah Tran, Zhirong Bao, Bruce Bowerman

P-173 Genetic basis of natural variation in X chromosome nondisjunction in *C. elegans*

Jun Kim, Jiseon Lim, Yeeun Yoon, Junho Lee

P-175 Regulation of ERM proteins in cortical membrane specialization

Joao Ramalho, Mike Boxem

P-177 Molecular mechanisms of developmentally controlled polyploidization in the *C. elegans* intestine

Lotte van Rijnberk, David Morgan, Matilde Galli

P-179 Second male meiotic division is independent on the canonical spindle assembly checkpoint signaling in *C. elegans*

Shang-Yang Chen, Jui-ching Wu

P-181 Activity and functional conservation of neuropeptide signaling in *C. elegans*

Victoria Groß, Miron Gershkovich*, Claudia Binder, Annette Beck-Sickinger, Torsten Schöneberg, Anette Kaiser, Simone Prömel

P-183 The THAP domain protein LIN-36 functions with the DREAM complex to repress transcription of proliferation promoting genes during quiescence

Csenge Gal, Alex Appert, Chiara Cerrato, Ni Huang, Julie Ahringer

P-185 Depletion of a neuronally expressed putative selenium binding protein ortholog induces stress resistance in *C. elegans*

Karl Köhnlein, Nadine Urban, David Guerrero-Gómez, Pavel Urbanek, Holger Steinbrenner, Christoph Kaether, Martin Srayko, Antonio Miranda-Vizuete, Lars-Oliver Klotz

P-187 DAF-16 produces a fold-change, not an absolute effect, on *C. elegans* survival under aging and stress.

Nicholas Stroustrup

P-189 Temporal scaling during *C. elegans* post-embryonic development

Olga Filina, Rik Haagmans, Jeroen van Zon

P-191 RNA Binding Proteins in stress resistance – a screen in *C. elegans*

Reza Esmaillie, Tim Krüger, Michael Ignarski, Rene Neuhaus, Francesca Fabretti, Roman-Ulrich Müller

P-193 Characterization of the C.elegans ubiquitin-modified proteome (ubiquitinome)

Batool Ossareh-Nazari, Anthi Katsiarimpa, Luis Briseno-Roa, Jorge Merlet, Lionel Pintard

P-195 Unbiased genetic screen to identify paternal factors involved in the regulation of the maternal mitochondrial transmission
Jihane Challita, Alice LeMorillon, Valeria Parrales, Aniela Zablocki, Jorge Merlet, Vincent Galy

P-197 The HIRA histone chaperone complex maintains normal cellular function in adult animals and protects against late-onset pleiotropic defects
Kirk Burkhart, Anna Corrionero, Steve Sando, Bob Horvitz

P-199 The Slit/Robo pathway component eva-1 and RhoGAP-containing domain gene 2RSSE.1 are novel Wnt target genes required for termination of neuroblast migration in *C. elegans*
Lorenzo Rella, Euclides E. Fernandes Pòvoa, Annabel L.P. Ebbing, Marco C. Betist, Hendrik C. Korswagen

P-201 Deciphering the role of mitochondrial prohibitins as lifespan modulators of sgk-1mutants
Mercedes M. Pérez-Jiménez, Blanca Hernando-Rodríguez, Mª Jesús Rodríguez-Palero, Antoni Pla, Roxani Gatsi, Marta Artal-Sanz

P-203 RAB-6.2 regulates LET-23 EGFR-mediated vulval induction
Sarah Gagnon, Kimberley Gauthier, Christian Rocheleau

P-205 Stable maintenance of neuronal cell fate by the che-1 genetic switch
Joleen J.H. Traets, Jeroen S. van Zon

P-207 Branched actin regulates Cadherin/HMR-1 trafficking
Sofya Borinskaya, Shashikala Sasidharan, Martha Soto

P-209 RNA Polymerase II CTD serine 2 phosphorylation regulates developmental arrest in *C. elegans*
Fanelie Bauer, Clement Cassart, Carlo Yague-Sanz, Francesca Palladino, Valerie Robert, Damien Hermand

P-211 Crosstalk between the ARP2/3 Complex and Formin Ensures Timely Cytokinesis
Fung-Yi Chan, Ana M. Silva, Joana Saramago, Ana X. Carvalho

P-213 Adapting a proximity labeling technique to identify novel non-centrosomal MTOC proteins in *C. elegans*
Ariana D. Sanchez, Tess Branon, Alice Y. Ting, Jessica L. Feldman

P-215 Identification and characterization of a fertilization signal pathway for female meiosis in *C. elegans*.
Rudra Banerjee, Jens Herzog, Martin Srayko

P-217 Characterization of the role(s) of the *C. elegans* Ceh-6 and Sox-2 transcription factors during a cellular transdifferentiation event.
Anne Daulny, Arnaud Ahier, David Rodriguez, Thomas Le Gal, Elena Morganti, Joshua Brickman, Sophie Jarriault

P-219 Dissecting the contribution of microRNAs to nervous system development and function
Chiara Alberti, Jingkui Wang, Luisa Cochella

P-221 Unravelling Actin-Filament Crosslinkers diversity in vivo
Ana Filipa Sobral, Fung Yi Chan, Ana Xavier Carvalho

P-223 Characterization of helminth complexes I and II of the ETC and analysis of their relevance in malate dismutation
Lucía Otero, Cecilia Martínez, Exequiel Barrera, Sergio Pantano, Gustavo Salinas

P-225 The Adhesion GPCR LAT-1 controls oocyte maturation and sperm guidance in *C. elegans*
Daniel Matúš, Franziska Fiedler, Julia Luterán, Claudia Binder, Torsten Schöneberg, Simone Prömel

P-227 Using designer receptors (DREADDs) to decipher G protein signalling pathways in *C. elegans*
Franziska Fiedler, Jana Winkler, Claudia Binder, Torsten Schöneberg, Doreen Thor, Simone Prömel

P-229 Developmental dynamics of gene expression and alternative polyadenylation in the Caenorhabditis elegans germline
Sean West, Desirea Mecenas, Michelle Gutwein, David Aristizabal-Corrales, Fabio Piano, Kristin Gunsalus

P-231 LIN-10 can promote LET-23 EGFR signalling independently of LIN-2 and LIN-7
Kimberley Gauthier, Christian Rocheleau

P-233 An artificial eggshell for the study of mechanical functions of eggshell in *C. elegans* development
Akiko Hatakeyama, Asako Sato, Yo Tanaka, Shuichi Onami

P-235 Germline signals repress signalling and metabolic programmes in *C. elegans*
Abraham Mains, Janna Hastings, Boo Virk, Olivia Casanueva

P-237 Involvement of epigenetic factors and metabolism in pluripotency maintenance in *C. elegans*
Francesca Coraggio, Ringo Püschel, Alisha Marti, Peter Meister

P-239 A novel model to visualize and quantify amyloid-beta fibrilization in vivo
Christian Gallrein, Manuel Iburg, Chetan Poudel, Sara Wagner-Valladolid, Gabriele Kaminski Schierle, Janine Kirstein

P-241 Dissecting the mechanism of transcriptional priming, a strategy for cellular diversification
Julien Charest, Thomas Daniele, Jingkui Wang, Luisa Cochella

P-243 The scent of a smoking gun: characterizing a new oomycete pathogen of *C. elegans*
Michael Fasseas, Clara Essmann, Michalis Barkoulas

P-245 Impairment of the DLK-1 MAP Kinase Pathway Suppresses p25α Induced Neurodegeneration of Dopaminergic Neurons in *C. elegans*.
Anders Olsen, Marie Fuglsang, Katrine Stenz, Freja Sørensen, Katrine Vogt, Lotte Vestergaard, Maria Doitsidou, Frederik Vilhardt, Poul Henning Jensen

P-247 Dissecting dynactin's role in bipolar spindle assembly in somatic cells of the *C. elegans* early embryo
Tania M. Silva, Patrícia A. Simões, Reto Gassmann

P-249 LIN-15B is necessary for enrichment of H3K9me2 on promoters of a subset of germline expressed genes in somatic cells
Andreas Rechtsteiner, Meghan Fealey, Thea Egelhofer, Susan Strome, Lisa Petrella

P-251 Sexy learning in *C. elegans*: Integration of conflicting experiences
Laura Molina-Garcia, Sergio Benavides-Laconcha, Arantza Barrios

P-253 In search of higher-order genetic interactions in Caenorhabditis elegans
Katarzyna Toch, Marta Labocha

P-255 4D lineage screen for regulators of neuroblast identity: cell size and proneural gene expression
Thomas Mullan, Terry Felton, Nadin Memar, Osama Kasem, Justina Yueng, Ralf Schnabel, Richard Poole

P-257 Exploring the Role of the Endoplasmic Reticulum Unfolded Protein Response (UPR-ER) in *C. elegans* Neurons
Nesem Ozbey, Christel Krueger, Soudabeh Imanikia, Ming Sheng, Olivia Casanueva, Rebecca Taylor

P-259 Using *C. elegans* as a model for studying telomere maintenance pathways
Karim Hussain, Helder Ferreira

P-261 An actin-dependent spindle positioning mechanism in the zygote of *Pristionchus pacificus*
Satoshi Namai, Daichi Sasaki, Asako Sugimoto

P-263 Understanding a mechanism of incomplete penetrance of human tumor suppressor gene PTEN by adaptive evolution of c. elegance
Anna Mellul, Irene Guberman, Idit Bloch, Yuval Tabach

P-265 Finding the Evolutionary Controls of Satiety Quiescence Behaviour using CeNDR
Bertalan Gyenes, André Brown

P-267 Characterization of a temperature-sensitive allele of egg-3
Amber Krauchunas, Kendall Flanagan, Peter Schweinsberg, Barth Grant, Andrew Singson

P-269 The effect of genetic background on seam cell development

Sneha Latha Koneru, Michalis Barkoulas

P-271 Tissue-specific ChIP-seq to study genomic distribution of Histone variants and Trasncription Factors

I. Selman Bulut, Baris Tursun

P-273 Are mitochondrial DNA deletion or protein aggregation determinants of lifespan in Caenorhabditis elegans?

Zhuangli Yee, Lakshmi Narayanan Lakshmanan, Rudiyanto Gunawan, Barry Halliwell, Jan Gruber

P-275 Transcriptional regulation by vitamin B12 of different metabolic pathways

Gabrielle Giese, Marian Walhout

P-277 Genetic dissection of neuropeptide cell biology at high and low activity in a defined sensory neuron.

Patrick Laurent, et al.

P-279 Syntaxin 7 Promotes Actin Localization During Spermatid Cell Division

Kristin Fenker, Linda Nikolova, Gillian Stanfield

P-281 Autophagy: load-bearer of *C. elegans*' metabolic changes

Ludovico Martins Alves, Christian Pohl

P-283 In vivo luminescent ATP *C. elegans* sensor strains for drug discovery in age-related diseases – an update.

Cristina Lagido

P-285 Dopaminergic modulation by quercetin: in silico and in vivo evidences

Daiana Avila, Willian Salgueiro, Fávero Paula, Rafaela Rios-Anjos, Michael Aschner

P-287 Uniaxial loading induces a scalable switch in cortical actomyosin flow polarization and reveals mechanosensitive components of cell division

Christian Pohl, Deepika Singh, Devang Odedra

P-289 The Exocyst Complex Promotes Germline Stem Cell Proliferation by Regulating the Trafficking of Notch Receptor in Caenorhabditis elegans

Pushpa Kumari, Harsh Kumar, Sivaram Mylavarapu

P-291 Overexpression of Werner syndrome helicase extends lifespan and healthspan in *C. elegans*

Hayley Lees, Sara Maxwell, Lynne Cox, Alison Woollard

P-293 IDENTIFICATION OF GENES INVOLVED IN THE INFERTILITY OF INSULIN MUTANTS

Cristina Viera-Osorio, Carlos Lopez-Viso, Sara González-Hernández, Manuel J. Muñoz-Ruiz, Marta Artal-Sanz, et al.

P-295 IDENTIFICATION OF NATURAL EXTRACTS THAT AFFECT GONADS DEVELOPMENTAL ARREST OF INSULIN/IGF MUTANTS.

Carlos López Viso, Manuel Alaiz Barragán, Ana María Brokate Llanos, Andrés Garzón Villar, Julio Girón Calle, Javier Vioque Peña, Manuel Jesús Muñoz Ruiz

P-297 Using *C. elegans* to investigate natural variation in protein mis-folding and related diseases

Yu Nie, Yiru Wang, Simon Harvey

P-299 The transcription factors LIN-31 and LIN-1 play a role in toroid formation during morphogenesis of the *C. elegans* vulva

L.M. Miller, A. Hajnal

P-301 The coiled-coil protein PCMD-1 organizes the assembly of the centrosome matrix

Anna C. Erpf, Nadin Memar, Ralf Schnabel, Mikeladze-Dvali Tamara

Poster session II

P-02 Force transmission between three tissues controls planar polarity establishment and embryonic morphogenesis
Ghislain Gillard, Ophélie Nicolle, Thibault Brugière, Sylvain Prigent, Mathieu Pinot, Grégoire Michaux

P-04 Interacting partners of BLMP-1 as a transcriptional activator in *C. elegans*
Hei Tung Michelle Fong, Takao Inoue, Thilo Hagen

P-06 Chryseobacterium nematophagum: A novel matrix digesting bacilli with an eclectic nematode diet.
Antony Page, Marie-Anne Felix, Mark Roberts

P-08 Quantitative RNA-seq meta analysis of alternative exon usage in *C. elegans*.
Denis Dupuy, Nicolas Tourasse

P-10 The Small GTPase RAC1/CED-10 Is Essential in Maintaining Dopaminergic Neuron Function and Survival Againstα-Synuclein-Induced Toxicit
Esther Dalfo, et al.

P-12 Lamin plays with SUN
Lenka Hůlková, Jana Rohožková, Pavel Hozák

P-14 Quantitative phenotyping and modeling identifies key behavioral rules underlying *C. elegans* aggregation
S Serena Ding, Linus Schumacher, Robert Endres, Andre Brown

P-16 A GFP::OB fold gene promoter fusion screen aim to identify cytosolic sensor receptor of viral immunity reveal the involvement of DNA licensing pathway components
Priyanka Mishra, Sheliza Shivji, Megan Cornell, Brandon Kong, Victoria Kooner, Albert Luu, Gabriel Lim, Frederic Pio

P-18 Regulation of DNA repair pathways to ensure gamete quality
Erika Rosenkranse, Carolyn Turcotte, Julia Laibach, Aidan Nowakowski, Richard Monsky, Nicolas Andrews, Paula Checchi

P-20 Unraveling the molecular mechanisms controlling monoaminergic neurons development through an RNAi screen
Ángela Jimeno-Martín, Miren Maicas Irigarai, Rebeca B. Ruiz, Nuria Flames Bonilla

P-22 Imidacloprid-containing pesticides disrupt *C. elegans* development
Beatrix Bradford, Paula Checchi

P-24 Some maternal mRNA transcripts are not homogenously distributed in early *C. elegans* embryos
Dylan M Parker, Marc T Nishimura, Sam Boyson, Erin Osborne Nishimura

P-26 EMR-1/emerin is involved in tissue-specific anchoring of chromatin to the nuclear envelope and neuromuscular junction activity
Celia M. Muñoz Jiménez, Cristina Ayuso, Agnieszka Dobrzynska, Peter Askjaer

P-28 Longevity and its transgenerational inheritance is enabled by repressive chromatin
Teresa Lee, Amanda Engstrom, David Katz

P-30 The subapical intermediate filament-rich endotube responds to and protects against microbial insults and toxins in the *C. elegans* intestine
Florian Geisler, Richard A. Coch, Christine Richardson, Martin Goldberg, Olaf Bossinger, Rudolf E. Leube

P-32 Identification of protein-protein interactions at the nuclear envelope
Agnieszka Dobrzynska, Javier Macías-León, Triana Solís-Vázquez, Carmen Espejo Serrano, Cristina Ayuso, Peter Askjaer

P-34 Cross-talk between the NOTCH and hypoxia response pathways modulates RAS/MAPK-mediated cell fate decisions in *C. elegans*
Sabrina Maxeiner, Judith Grolleman, Tobias Schmid, Jan Kammenga, Alex Hajnal

P-36 Embryonic Exposure to Amphetamine Reduces Gene Expression of the Dopamine Transporter

Lucia Carvelli, Ganesh Ambigapathy, Talus McCowan, Archana Dhasarathy

P-38 piChIP: a single locus IP technology to identify novel factors in the *C. elegans* germline Nuclear RNAi pathway

Ahmet Can Berkyurek, Guilia Furlan, Alper Akay, Fabian Braukmann, Peter Dimaggio, Eric Miska

P-40 Modeling human RASopathies in vulval development to dissect the molecular mechanisms and identify novel disease-genes

Luca Pannone, Simona Coppola, Emanuela Pone, Francesca Pantaleoni, Ivan Gallotta, Elia Di Schiavi, Marco Tartaglia, Simone Martinelli

P-42 Generation and validation of a microfluidic platform for high-throughput detection of cancer metabolites in urine samples

Martina Di Rocco, Enrico Lanza, Davide Caprini, Luca Pannone, Simone Martinelli, Giancarlo Ruocco, Viola Folli

P-44 A novel correction mechanism regulates nuclear position and ensures proper DNA segregation during late cytokinesis

Anne Pacquelet, Matthieu Jousseaume, Grégoire Michaux

P-46 Long-term monitoring of cytosolic and mitochondrial Ca2+ dynamics in *C. elegans* pharynx

Pilar Álvarez, Paloma Garcia-Casas, Jessica Arias-del-Val, Adolfo Sanchez-Blanco, Rosalba I Fonteriz, Javier Alvarez, Mayte Montero

P-48 The identification and characterization of chromatin regulators involved in coelomocyte to neuron conversion in *C. elegans*

Ismail Özcan, Anna Reid, Margaux Quiniou, Andreas Ofenbauer, Baris Tursun

P-50 Importance of the glutamate synthase homologue (W07E11.1) for lifespan extension and stress resistance in the *C. elegans* daf-2 mutant

Aleksandra Zečić, Bart Braeckman

P-52 Loss of glutathione redox homeostasis impairs proteostasis by collapse of autophagy-dependent protein degradation

David Guerrero-Gómez, José Antonio Mora-Lorca, Beatriz Sáenz-Narciso, Francisco José Naranjo-Galindo, Fernando Muñoz-Lobato, Christopher D. Link, Christian Neri, Rafael Vázquez-Manrique, Peter Askjaer, Juan Cabello, Antonio Miranda-Vizuete

P-54 Identification of potent drug candidates for attenuation of Cisplatin-induced neurotoxicity in the model organism *C. elegans*

Anna Wellenberg, Lea Weides, Julia Bornhorst, Barbara Crone, Uwe Karst, Gerhard Fritz, Sebastian Honnen

P-56 A natural molecular variant enhancing *C. elegans* dauer formation in response to diverse environmental cues

Bénédicte Billard, Paul Vigne, Clotilde Gimond, Christian Braendle

P-58 Insulin-dependent quiescence and arrest at hatching

Bruce Wightman

P-60 A link between *C. elegans* morphogenesis and mRNA export.

Angelina Zheleva, Eva Gomez-Orte, Beatriz Saenz-Narciso, Begoña Ezcurra, Maria de Toro, Henok Kassahun, Hilde Nilsen, Ralf Schnabel, Juan Cabello

P-62 Effect of the diet type and temperature on the *C. elegans* transcriptome

Eva Gómez-Orte, Eric Cornes, Angelina Zheleva, Beatriz Saenz-Narciso, Maria de Toro, Maria Iniguez, Rosario Lopez, Begoña Ezcurra, Adolfo Sanchez-Blanco, Julian Ceron, Juan Cabello

P-64 Actomyosin contractility regulators stabilize the cytoplasmic bridge of the primordial germ cells Z2 and Z3 during *C. elegans* embryogenesis.

Eugénie Goupil, Rana Amini, David H. Hall, Jean-Claude Labbé

P-66 spe-51 (as39) is dispensable for early spermatogenesis and spermiogenesis but required for sperm function in *C. elegans*
Xue Mei, Gunasekaran Singaravelu, Marina Druzhinina, Sunny Dharia, Andrew Singson

P-68 Deciphering the role of centrosomes in breaking symmetry of the *C. elegans* zygote
Kerstin Klinkert, Coralie Busso, Sarah Herrman, Lukas von Tobel, Pierre Gönczy

P-70 Splicing-related Retinitis Pigmentosa mutations in *C. elegans*
Dmytro Kukhtar, Karinna Rubio-Peña, Xènia Serrat, Julián Cerón

P-72 Uncovering mechanisms of centriole elimination during *C. elegans* oogenesis
Marie Pierron, Pierre Gönczy

P-74 Predicting individual differences in viral susceptibility caused by natural genetic variation within species
Lisa van Sluijs, Mark G. Sterken, Yiru Wang, Wannisa Ritmahan, Mitra L. Gultom, Joost A. G. Riksen, Rita J. M. Volkers, L. Basten Snoek, Gorben P. Pijlman, Jan E. Kammenga, et al.

P-76 Transcriptional regulatory logic of neuronal ciliogenesis in *C. elegans*
Rebeca Brocal Ruiz, Nuria Flames

P-78 Notch independent functions of LAG-1/RBPJ in the selection of ADF chemosensory neuron fate
Miren Maicas, Ángela Jimeno, Mark Alkema, Nuria Flames

P-80 Dithiolic glutaredoxins in Caenorhabditis elegans
Milena Lubisch, Dirk Stegehake, Anne Kaminski, Eva Liebau

P-82 Inhibition of Sarco-Endoplasmic Reticulum Ca2+ ATPase extends the lifespan in *C. elegans* worms
Paloma García Casas, Jessica Arias Del Val, Pilar Alvarez Illera, Rosalba I Fonteriz, Mayte Montero, Javier Alvarez

P-84 The role of neuronal DEG/ENaC ion channel family members in organismal stress responses
Dionysia Petratou, Nektarios Tavernarakis

P-86 Identification of genetic suppressors of smn-1 in neurodegeneration.
Pamela Santonicola, Ivan Gallotta, Alessandro Esposito, Giuseppina Zampi, Elia Di Schiavi

P-88 Robustness in the transcriptional programs that maintain neuronal cell fates
Konstantina Filippopoulou, Guillaume Bordet, Carole Couillault, Vincent Bertrand

P-90 The role of developmental genetic architecture in shaping evolutionary trends
Joao Picao-Osorio, Christian Braendle, Marie-Anne Félix

P-92 The Pre-Replication Complex Governs the Invasive Cell Fate of the Caenorhabditis Elegans Anchor Cell in a Replication-Independent Manner
Evenlyn Lattmann, Ting Deng, Vibhu Prasad, Charlotte Lambert, Michael Daube, Ossia Eichhoff, Urs Greber, Reinhard Dummer, Mitch Levesque, Alex Hajnal, et al.

P-94 Intestinal polarity is maintained by V0-ATPase-dependent apical sorting in *C. elegans*
Aurelien Bidaud-Meynard, Ophélie Nicolle, Gregoire Michaux

P-96 Acute drug responses in *C. elegans* reveal complex novel biology
Andy Fraser, Mark Spensley, Sam Dell Borrello, Margot Lautens, Taylor Davie

P-98 From the transcriptome to the metabolome: A systems view on the impact of the mitochondrial prohibitin complex during aging.
Artur Bastos Lourenço, Francisco J García-Rodríguez, Karl Burgess, Christoph Kaleta, Marta Artal Sanz

P-100 Activation of insulin signaling induces cell divisions in Caenorhabditis elegans L1 arrest.
Ian Chin-Sang, Shanqing Zheng, Jeffrey Boudreau

P-102 Identification of Heterotrimeric G-proteins Involved in the Modulation of the Exogenous RNA Interference Response by IP3 Signalling in *C. elegans*
Alice Rees, Howard Baylis

P-104 EXC-4/CLIC and G-protein signaling during tubulogenesis in *C. elegans*
Anthony Arena, Dan Shaye

P-106 Regulation of Caenorhabditis elegans neuronal function by the putative oxaloacetate decarboxylase FAHD-1
Giorgia Baraldo, Hildegard Mack, Pidder Jansen-Dürr

P-108 Cell-cell fusion of chemosensory neurons alters the animal's response to odours.
Rosina Giordano-Santini, Eva Kaulich, Massimo A. Hilliard

P-110 The TRIM32 protein GRIF-1 controls developmental proteolysis of GLD-2 cytoPAP
Tosin Oyewale, Christian Eckmann

P-112 Differences in Endogenous Proteasome Expression in *C. elegans*
Elisa Mikkonen, Caj Haglund, Carina I Holmberg

P-114 A transcription factor collective defines the HSN serotonergic neuron regulatory landscape
Carla Lloret-Fernández, Miren Maicas, Carlos Mora-Martínez, Alejandro Artacho, Ángela Jimeno-Martín, Laura Chirivella, Peter Weinberg, Nuria Flames

P-116 C.elegans progenitor cell fate control through the EGR-type transcription factor LIN-29
Chiara Azzi, Florian Aeschimann, Anca Neagu, Helge Grosshans

P-118 C elegans high content behavioural screening for drug repositioning
Ida Barlow, Adam McDermott-Rouse, Oliver Howes, Andre Brown

P-120 Stress-induced cellular surveillance and behavioural responses exhibit a complementary pattern in Caenorhabditis elegans
Eszter Gecse, Beatrix Gilányi, Márton Csaba, Csaba Sőti

P-122 Effects of asymmetric cell division on regulation of cell migration
Erik Schild, Shivam Gupta, Andrew Mugler, Hendrik Korswagen

P-124 Protective effect of plant hormones cytokinins in Caenorhabditis elegans
Alena Kadlecová, Tomáš Jirsa, Ondřej Novák, Martin Hönig, Lucie Plíhalová, Karel Doležal, Miroslav Strnad, Jiří Voller

P-126 The interneuron RIS and lethargus in *C. elegans*
Elisabeth Maluck, Henrik Bringmann

P-128 The role of the HOX protein LIN-39 in the regulation of VPC proliferation
Svenia Heinze, Alex Hajnal

P-130 Physical and functional interaction between the SET1/COMPASS component CFP-1/CXXC and the Sin-3S/HDAC complex at promoter regions
Cecile Bedet, Flore Beurton, Matthieu Caron, David Cluet, Marion Herbette, Hélène Polvèche, Przemyslaw Stempor, Yohann Couté, Alex Appert, Julie Ahringer, Francesca Palladino

P-132 Decoding the rules for PATC-mediated prevention of gene silencing in *C. elegans* germline
Monika Priyadarshini, Christian Froekjaer-Jensen

P-134 The *C. elegans* Aryl-Hydrocarbon Receptor (AHR-1) has evolutionary conserved functions and influences healthy ageing
Vanessa Brinkmann, Alfonso Schiavi, Anjumara Shaik, Lisa Tschage, Ralph Menzel, Natascia Ventura

P-136 Pro-longevity mitochondrial stress prevents *C. elegans* germline apoptosis through BRCA1/BARD1 tumor suppressor genes
Alfonso Schiavi, Alessandro Torgovnick, Henok Kassahun, Anjumara Shaik, Silvia Maglioni, Shane Rea, Sebastian Honnen, Björn Schumacher, Hilde Nilsen, Natascia Ventura

P-138 A mitochondrial isocitrate dehydrogenase prevents direct reprogramming of germ cells to neurons in *C. elegans*
Nida ul Fatima, Ena Kolundzic, Anna Reid, Baris Tursun

P-140 Channel Nucleoporins recruit the Polo-like kinase PLK-1 to the Nuclear Pore Complexes in prophase to direct Nuclear Envelope Breakdown in *C. elegans* embryos

Lionel Pintard, Lisa Martino, Stéphanie Morchoisne-Bolhy, Dhanya Cheerambathur, Lucie Van Hove, Julien Dumont, Nicolas Joly, Arshad Desai, Valerie Doye

P-142 mTOR complex 2 negatively regulates autophagy via Serum- and Glucocorticoid-inducible Kinase (SGK-1) independent of DAF-16/FoxO

Helena Aspernig, Wenjing Qi, Antje Thien, Ralf Baumeister

P-144 Role of KDM8/Jmjd-5 in preserving genome stability and germ line immortality

Nico Zaghet, Pier Giorgio Amendola, Anna Elisabetta Salcini

P-146 AFF-1-coated pseudoviruses as a novel tissue-specific delivery system can improve neuronal regeneration by fusion in *C. elegans*

Anna Meledin, Benjamin Podbilewicz

P-148 Genetic Control of the Maintenance of AIA Cell Identity

Josh Saul, Takashi Hirose, Bob Horvitz

P-150 The activity of unc-1/stomatin-like protein in the nervous system modulates protein homeostasis in *C. elegans*.

Ana Pilar Gómez Escribano, Jose Bono Yagüe, Irene Real Arévalo, Qiuyi Cheng Zang, Jose Blanca, Maria Dolores Sequedo Pérez, Joaquín Cañizares, Juan Burguera, Carmen Peiró, José Maria Millán Salvador, Rafael Vázquez Manrique

P-152 Gene expression profiles and alpha-synuclein toxicity varys in a *C. elegans* model of PD with variable natural genetic backgrounds

Yiru Wang, Basten Snoek, Mark Sterken, Joost Riksen, Jana Stastna, Yu Nie, Jan Kammenga, Simon Harvey

P-154 A pseudogene going astray: Is the transdifferentiation inhibitor F55A3.7 a novel non-coding RNA?

Andreas Ofenbauer, Ena Kolundžić, Alexander Gosdschan, Baris Tursun

P-156 Sleep counteracts aging phenotypes to promote survival of starvation

Henrik Bringmann, Florentin Masurat, Yin Wu, Jasmin Preis

P-158 Genetic analysis of a sas-6 mutant reveals mitotic and meiotic differences in centriole duplication and stability

Kevin O'Connell, Nicole DeVaul, Gunar Fabig, Kevin O'Connell

P-160 Modeling rare monogenic human diseases in *C. elegans*

Andy Golden, Ben Nebenfuehr, Carina Graham, Tyler Hansen, Peter Kropp, Isabella Zafra-Martinez

P-162 Single-Molecule Real Time sequencing reveals large structural variants in natural isolates of *C. elegans*

Cristian Riccio, Martin Hemberg, Eric Miska

P-164 Unequal distribution of centrosomal lin-5 mRNA during asymmetric division of *C. elegans* embryos

Alexandra Bezler, Zoltán Spiró, Pierre Gönczy

P-166 A Zona Pellucida domain protein and Patched-related protein promote apical ECM assembly.

Jennifer Cohen, Rachel Forman-Rubinsky, Meera Sundaram

P-168 TORC1 hyperactivation effects in Caenorhabditis elegans' nervous system

Aikaterini Stratigi, Patrick Laurent

P-170 *C. elegans* blastomeres clear the corpse of the second polar body by LC3-associated phagocytosis

Gholamreza Fazeli, Maurice Stetter, Jaime Lisack, Ann Wehman

P-172 A forward genetic screen to identify temperature-sensitive, early cell division mutants

Thalia Padilla, Nick Jackson, Bruce Bowerman

P-174 Modulation of FMRF like neuropeptides genes (flps) transcription by neuroendocrine signalling in *C. elegans*

J. Ramiro Lorenzo, Aikaterini Stratigi, Patrick Laurent

P-176 IDENTIFICATION OF LOW-DENSITY LIPOPROTEIN RECEPTOR-RELATED 8 INVOLVEMENT IN ALZHEIMER'S DISEASE
Carla Bertapelle, Alessandro Medoro, Donatella Mignogna, Claudio Russo, Elia Di Schiavi

P-178 The LSL-1 zinc-finger protein promotes meiotic prophase progression
David Rodriguez Crespo, Chantal Wicky

P-180 A *C. elegans* model for the histone H3.3K27M cancer driver mutation
Kamila Delaney, Maude Strobino, Joanna M. Wenda, Florian A. Steiner

P-182 Natural variation in the genetic architecture of a germ stem cell niche
Sarah Fausett, Christian Braendle

P-184 Analysis of inter-individual transcriptional variability of stress response genes in *C. elegans* by high-throughput qRT-PCR in single worms
Laetitia Chauve, Catalina Vallejos, Janna Hastings, John Marioni, Olivia Casanueva

P-186 Investigating the molecular mechanisms of SET-24 in maintaining germline immortality
Giulia Furlan, Lise Frézal, Ahmet Can Berkyurek, Alper Akay, Marie-Anne Felix, Eric Miska

P-188 Investigating the mechanism of chromatin domain formation
Garima Sharma, Chiara Cerrato, Ni Huang, Julie Ahringer

P-190 Evolution of QR neuroblast migration and mig-1 regulation in *C. elegans* and other nematodes.
Clement Dubois, Marie-Anne Felix

P-192 Analysis of mechanisms underlying the metabotropic signal mediated by the Adhesion GPCR LAT-1 in oriented cell division
Lidia Duplice Simone Prömel, et al.

P-194 Unraveling the regulatory network of a complete *C. elegans* neural lineage
Euclides Fernandes Póvoa, Annabel Ebbing, Lorenzo Rella, Marco Betist, Hendrik Korswagen

P-196 Characterizing mechanisms of neuronal plasticity at single-cell resolution in the dauer-exiting Caenorhabditis elegans larva
Friedrich Preußer, Ella Bahry, Stephan Preibisch

P-198 Investigating the role of Calcium signaling mediators in Caenorhabditis elegans lifespan regulation by signals from the germline
Laura Buck, James Moresco, John Yates, Cynthia Kenyon, Hildegard Mack

P-200 Germline stem cells behaviour and differentiation in filarial nematodes, two processes under the endosymbiont bacteria Wolbachia control
Mercedes M. Pérez-Jiménez, Vincent Foray, Nour Fattouh, Frederic Landmann

P-202 Genetic mechanisms regulating TWK-28, a potassium channel controlling the locomotion of Caenorhabditis elegans
Noura Zariohi, Marie Gendrel, Alice Leclercq-Blondel, Alice Peysson, Thomas Boulin

P-204 Investigating RACK-1's role in regulating stem cell proliferation in the *C. elegans* germ line
Kara Vanden Broek, Chris Wang, Xin Wang, Dave Hansen

P-206 Insulin signaling and the age of the mothers modulate L1 arrest and recovery
María Olmedo, Alejandro Mata-Cabana, María Jesús Rodriguez-Palero, Antonio Fernández-Yáñez, Sabas García-Sánchez, Martha Merrow, Marta Artal-Sanz

P-208 A new, general regulator of programmed cell death in *C. elegans*
Nadin Memar, Ryan Sherrard, Ralf Schnabel, Barbara Conradt

P-210 Peroxisomal Retrograde Signaling in Caenorhabditis elegans
Stéphane G. Rolland, Elisabeth Rackles

P-212 Novel role for GSA-1, a heterotrimeric G protein alpha subunit, during ovulation in the *C. elegans* spermatheca
Perla Castaneda, Erin Cram

P-214 An optogenetic arrhythmia model to study catecholaminergic polymorphic ventricular tachycardia mutations
Marcial Alexander Engel, Elisabeth Fischer, Alexander Gottschalk, Christina Schüler

P-216 Tissue-specific degradation of essential centrosome components reveals distinct microtubule populations at microtubule organizing centers
Maria Sallee, Jennifer Zonka, Taylor Skokan, Brian Rafetry, Jessica Feldman

P-218 A regulatory network of the GATA factor elt-1, Hox genes ceh-13, nob-1, and Wnt effectors pop-1 and sys-1 regulates early lineage patterning in *C. elegans* embryos
Amanda L. Zacharias, Elicia Preston, Teddy D. Lavon, Barrington Alexander Bennett, Shaili D. Patel, Ilona Jileaeva, John Isaac Murray

P-220 Atlas of cell shapes, actomyosin activity and cell-cell adhesion in *C. elegans* early embryogenesis.
Francesca Caroti, Rob Jelier

P-222 A short helix in the C-terminal region of dynein light intermediate chain links the motor to structurally diverse adaptors for cargo transport
Cátia Carvalho, Ricardo Celestino, José Bernardo Gama, Reto Gassmann

P-224 *C. elegans* as a model to investigate mitochondrial disorders
Valeria Morbidoni, Cristina Cerqua, Maria Andrea Desbats, Leonardo Salviati, Eva Trevisson

P-226 Inferring gene regulatory network governing early ageing process in *C. elegans*
Manusnan Suriyalaksh, Marta Sales Pardo, Nicolas Le Novere, Olivia Casanueva

P-228 Uncovering the role of the scaffold apical PAR proteins in established epithelial tissues
Helena Pires, Victoria G. Castiglioni, Jana Kerver-Stumpfova, Mike Boxem

P-230 M05D6.2, an ortholog of human T-complex protein 11 (TCP11), is necessary for sperm function and fertility in Caenorhabditis elegans
Emily Lopes, Amber Jacob, Danielle Cooley, Matthew Marcello

P-232 Cellular response to Wnt signals: an in vitro approach using *C. elegans* embryonic cells
Pritha Pai, Pierre Recouvreux, Pierre-Francois Lenne

P-234 An endomembrane-resident zinc transporter negatively regulates systemic RNAi in *C. elegans*.
Katsufumi Dejima, Rieko Imae, Yuji Suehiro, Shohei Mitani

P-236 Adhesion GPCRs in regulation of food intake and metabolism of *C. elegans*
Johanna Schön, Johanna Weinert, Daniel Matúš, Torsten Schöneberg, Simone Prömel

P-238 Examining promoter states at the single molecule level during transcription initiation
Jennifer Semple, Arnaud Krebs, Peter Meister

P-240 Epigenetic mechanisms of nematode mouth-form plasticity
Michael Werner, et al.

P-242 Investigating glia-to-neuron cell fate switches in *C. elegans*
Michele Sammut, Rachel Bonnington, Milou van der Lans, Kishan Khambhaita, David Elliott, Arantza Barrios, Richard Poole

P-244 Evolutionary Stable Strategies of Signalling and Signal Interference in Competitive Ephemeral Environments
Arthur Hills, Mark Viney, Simon Harvey

P-246 Rewiring of the germ line transcriptional network in *C. elegans* through TE co-option
Francesco Nicola Carelli, Chiara Cerrato, Jürgen Jänes, Julie Ahringer

P-248 Model-free analysis of spindle elongation trajectories in C.elegans one-cell embryo.
Yann Le Cunff, Laurent Chesneau, Sophie Theis, Valentin Costes, Sylvain Pastezeur, Jacques Pécréaux

P-250 H3K4 methylation and chromatin organization in the *C. elegans* germline.
Marion Herbette, David Llères, Aymeric Bailly, Robert Feil, Valérie Robert, Francesca Palladino

P-252 Nicotinamide-N-methyltransferase controls behavior, neurodegeneration and lifespan by regulating neuronal autophagy
Kathrin Schmeisser, Alex Parker

P-254 Phospholipid translocase function regulates vesicle transport in specific ciliated chemosensory neurons.
Simon Tuck, Lars Nilsson, Shapour Rahmani, Ani Minasian, Manon Baures

P-256 The physiological role of acd-5 in dauer larvae formation and chemosensation in *C. elegans*
Laura Grundy, Eva Kaulich, William R Schafer

P-258 Analysis of heterochromatin in repetitive element repression and ageing
Tessa Gaarenstroom, Alicia McMurchy, Przemyslaw Stempor, Alex Appert, Luke Harvey, Michael Schoof, Ni Huang, Andrea Frapporti, Matt Churgin, Christopher Fang-Yen, Julie Ahringer

P-260 Temporary Atresia and Corsetry are Essential for Worms to Molt
Hannah Maul-Newby, Chloe Maybrun, Alison Frand

P-262 MIG-6/papilin, an extracellular matrix protein, mediates the maintenance of neuronal architecture
Claire Bénard, Malika Nadour, Lise Rivolet, Andrea Thackeray

P-264 Shredding 3' UTRs
Jonathan Froehlich, Bora Uyar, Margareta Herzog, Altuna Akalin, Nikolaus Rajewsky

P-266 *C. elegans* early embryonic cytokinesis requires myosin motor activity
Joana Saramago, Daniel Osório, Fung Yi Chan, et al.

P-268 Proteostasis Regulation and Interplay
Carina I. Holmberg, Elisa Mikkonen, Sweta Jha

P-270 Computational Modeling of Early *C. elegans* Embryogenesis
Lidia Yamamoto, Rob Jelier

P-272 CREB mediates a developmental plasticity in *Caenorhabditis elegans*
Jisoo Park, Yongjin Chun, Sundong Pyo, Scott J. Neal, Rebecca A. Butcher, Piali Sengupta, Kyuhyung Kim

P-274 Evaluation of nanoparticles in vivo using the Caenorhabditis elegans model organism
Anna Laromaine, Laura Gonzalez-Moragas, Luo Zhongrui, Anna Roig

P-276 Mechanical sensing of damage?
Rémy Pujol, Chantal Cazevieille, Clara Essmann, David Hall, Jonathan Ewbank, Nathalie Pujol

P-278 Regulation of anterior fates by pop-1 and sys-1 in the *C. elegans* embryo
Jonathan Rumley, Amanda Zacharias, John Murray

P-280 Netrin acts redundantly with the conserved homeobox gene caudal/pal-1 to specify posterior patterning and axon guidance
Sophie Gilbert, Alison Woollard

P-282 Behavioural Screening to Predict Pesticide and Drug Mode of Action in *C. elegans*
Adam McDermott-Rouse, Andre Brown

P-284 The microRNA let-7 controls three distinct developmental events exclusively through the RNA-binding protein LIN41 and its four targets
Florian Aeschimann, Magdalene Rausch, Helge Großhans

P-286 The metabolic action of organoselenium compounds in C.elegans
Caroline Quines, Flávia Pereira, Cristina Nogueira, Gilson Zeni, Daiana Avila

P-288 Histone Acetylations and Transcription Facilitate De Novo Centromere Establishment
Jing Zhu, Kevin Chi Lok Cheng, Karen Wing Yee Yuen

P-290 Characterization of rme-8, a newly identified regulator of protein homeostasis

Mirjam Ax, Joanna Maus, Anna S Besemer, Christian von Hilchen, Heike Huesmann, Andreas Kern, Christian Behl, Albrecht M Clement

P-292 Microbial crystal proteins are not a worm's best friend

Hala Fahs, Fathima Refai, Robert White, Giselle Cipriani, Stephan Kremb, Glenn Butterfoss, Mireille Kallassy, Fabio Piano, Kristin Gunsalus

P-294 Characterization of a p150/DNC-1 mutant that uncouples dynactin's role in dynein recruitment from its role in dynein activation

Joana Duro, Daniel Barbosa, Reto Gassmann

P-296 Investigating the role of the ARF GTPase arf-3 in regulating seam cell development and secretion

Aidan Walker, Alison Woollard

P-298 Investigation of mechanism of ketamine-induced anti-depressant effects in *C. elegans*

Duygu Yücel

P-300 WormJam: A consensus *C. elegans* Metabolic Reconstruction and Metabolomics Community

Janna Hastings, Nicolas LeNovere, Michael Witting, Olivia Casanueva

P-302 Contractile ring-dependent cytoplasmic partitioning in non-mitotic germ cells

Chelsea Maniscalco, Jeremy Nance

ABSTRACTS

Wednesday, 13 June 2018 - Auditorium - 17:10 – 20:00

Session 1 Genomics & Systems Biology

S1-01 Genome architecture and regulation

Julie Ahringer

The Gurdon Institute, University of Cambridge

Genome regulation takes place in the context of chromatin, the organization of DNA with histones and hundreds of associated proteins and RNAs. Although the sequence of DNA is essentially the same in all cells, chromatin structure differs at local and domain scale levels to achieve correct patterns of active and repressed transcription. This regulation of chromatin structure plays a central role in normal development and its misregulation is associated with disease. I will discuss our work on different architectural features of the genome, such as regulatory elements, broad chromatin domains, and 3D genome organization.

S1-02 A comprehensive spatiotemporal map of embryonic gene expression

Qin Zhu[2], Hannah Dueck[1], Priya Sivaramakrishnan[1], Amanda Zacharias[1], Shaili Patel[1], Elicia Preston[1], Junhyong Kim[1], John Murray[1]

[1]*Department of Genetics, Perelman School of Medicine, University of Pennsylvania, USA*

[2]*Department of Biology, University of Pennsylvania, USA*

A central goal of developmental biology research is to identify how genes control cell fate and execute the complex cellular behaviors needed to generate a multicellular organism. A complete description of gene expression patterns across all cells of a developing organism would be a valuable resource to predict genes important for cell fate regulation and other developmental processes, facilitating mechanistic reverse genetic studies. We have adopted a “whole organism shotgun” approach to define temporal changes in mRNA levels genome-wide at single cell resolution across the embryo. We used the 10x Genomics platform to collect single cell RNA-seq data from over 15,000 single cells from mixed stage embryos between the ~50-cell and comma stages. In the early embryo, somatic cells share a common temporal pattern characterized by gradual decreases in maternal transcripts and expression of broadly expressed temporally specific zygotic transcripts. Lineage-specific transcripts represent a smaller fraction of the transcriptome but can be identified by integrating the scRNA-seq data with single-cell expression patterns derived from time-lapse microscopy. Zygotic gene expression in the intestine, muscle and hypodermis are characterized by a stereotyped temporal expression sequence with relatively little spatial heterogeneity. In contrast, the transcriptomes of neurons, glia and other specialized cell types are dominated by genes that are specific to each individual cell identity. Finally, the pharynx shares both signatures, with a strong common temporal program followed by highly distinct specialization of gene expression as distinct pharyngeal cell types differentiate. Integration TF binding data allows the inference of novel developmental regulators in each tissue. We developed an interactive program that allows users to explore expression of their genes of interest and to identify genes with variable expression within or between cell types.

S1-03 Spatially-resolved transcriptomics in *C. elegans* identifies sex-specific differences in gene expression patterns

Annabel L. P. Ebbing[1], Abel Vertesy[1], Marco Betist[1], Bastiaan Spanjaard[2], Jan Philipp Junker[2], Eugene Berezikov[3], Alexander van Oudenaarden[1], Hendrik C. Korswagen[1]

[1]*Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences and University Medical Center Utrecht, Uppsalalaan 8, 3584 CT, Utrecht, The Netherlands.*

[2]*Berlin Institute for Medical Systems Biology, Max Delbrück Center for Molecular Medicine, Berlin, Germany.*

[3]*European Research Institute for the Biology of Ageing, University of Groningen, University Medical Center Groningen, Groningen.*

To advance our understanding of the genetic programs that drive cell and tissue specialization, it is necessary to obtain a comprehensive overview of gene expression patterns. We have used RNA tomography to generate the first high-resolution, anteroposterior gene expression maps of *C. elegans* males and hermaphrodites. In brief, we cryo-sectioned a young adult animal into 20 μm thick sections along the anteroposterior axis. Next, each section was sequenced using the sensitive CEL-seq method, after which the sequencing data was aligned to create an anteroposterior gene expression map. Using this approach, an average of 16694 genes (>93% of genes identified in young adult animals by bulk RNA sequencing) could be detected along the length of the animal. As expected from the thin sectioning width, we found that the resolution of the expression maps is sufficient to detect individual cell and tissue types. Moreover, because of the relatively simple and invariant anatomy of *C. elegans*, expression maps from different animals could be precisely aligned and merged to facilitate the detection of lowly expressed genes. To explore the expression maps, we have developed computational approaches to identify cell and tissue specific genes. These methods enabled us to identify genes expressed in specific tissues, such as the germline, but also single neuron pairs in the head, demonstrating the sensitivity of our approach. For further validation, we focused on genes expressed in the male reproductive tract, which led to the identification of a novel group of secreted proteins that are required for male fertility. In summary, our results demonstrate that RNA tomography maps provide a powerful resource to identify novel sex- and tissue-specific gene functions in *C. elegans*. The expression maps and an interactive search tool to identify co-expressed genes will be made available through the project website.

S1-04 Quantitative RNA-seq meta analysis of alternative exon usage in *C. elegans*.

Denis Dupuy, Nicolas Tourasse

Université de Bordeaux, Inserm U1212, CNRS UMR5320, Institut Européen de Chimie et Biologie (IECB), 2, rue Robert Escarpit, 33607 Pessac, France.

Almost twenty years after the completion of the *C. elegans* genome sequence, gene structure annotation is still an ongoing process with new evidence for gene variants still being regularly uncovered by additional in-depth transcriptome studies. While alternative splice forms can allow a single gene to encode several functional isoforms the question of how much spurious splicing is tolerated is still heavily debated. We gathered a compendium of 1,682 publicly available *C. elegans* RNA-seq datasets to increase the dynamic range of detection of RNA isoforms and obtained robust measurements of the relative abundance of each splicing event. While most of the splicing reads come from reproducibly detected splicing events, a large fraction of purported junctions are only supported by a very low number of reads. We generated annotated gene models including quantitative exon usage information for the entire *C. elegans* genome. This allows users to visualize at a glance the relative expression of each isoform for their gene of interest that takes into account the expression level of each gene to discriminate robust splicing events from potential biological noise. We found that rarely used splice sites disproportionately come from highly expressed genes and are significantly less conserved in other nematode genomes than splice sites with a higher usage frequency. Our increased detection power also confirmed trans-splicing for at least 84% of *C. elegans* protein coding genes. The genes for which trans-splicing was not observed are overwhelmingly low expression genes, suggesting that the mechanism is pervasive but not fully captured by organism- wide RNA-Seq.

S1-05 Systematic analysis of cell cycle phases reveals only a single cell pair develops with full cell cycle during *C. elegans* embryogenesis

Ming-Kin Wong[1], Vincy Wing Sze Ho[1], Xiaotai Huang[2], Runsheng Li[1], Lu-yan Chan[1], Hon Chun Kaoru Ng[1], Hong Yan[3], Zhongying Zhao[1, 4]

[1]*Department of Biology, Hong Kong Baptist University, Hong Kong, China*

[2]*School of Computer Science and Technology, Xidian University, Xi'an, China*

[3]*Department of Electronic Engineering, City University of Hong Kong, Hong Kong, China*

[4]*State Key Laboratory of Environmental and Biological Analysis, Hong Kong Baptist University, Hong Kong, China*

Unlike cell cycles in yeast or a cultured cell that usually consist of four consecutive phases, i.e., G1, S, G2 and M, early embryonic cell cycles in most metazoans comprise S and M phases only with no gap phases. Precise knowledge on systematic introduction of the gap phases in a developing embryo is essential for understanding how cell-cycle progression is coupled with cell fate specification. However, developmental introduction of gap phases remains mysterious. To develop a systems view of developmental stage- or cell fate-dependent introduction of cell cycle gap phases, we generate multiple fluorescence ubiquitin cell cycle indicators (FUCCI) in *C. elegans* using the degrons derived from either CDT-1 or CYB-1, which are rendered ubiquitously expressed in the nuclei of nearly all cells. Time-lapse 3D imaging followed by automated cell lineaging reveals a clear expression dynamics of the reporters as expected for every cell with one-minute interval, allowing precise demarcation of cell cycle phases. Surprisingly, lineaging up to roughly three-fold stage reveals that only a single cell pair develops with full cell cycle throughout embryogenesis, whereas the remaining cells except E lineage do not acquire any gap phase until they initiate their last round of division, during which CDT-1 degron starts to accumulate, indicating cell cycle arrest in G0/G1 phase. E lineage appears to acquire G2 phase in E2 and E4 stage but follows the same patterns as other cells at E20 stage. The results indicate that the asynchrony between sister cells is primarily dictated by the S phase duration. The degrons also demonstrate expression dynamics in postembryonic tissues including germline. In summary, a worm version of FUCCI reveals important biology of embryonic cell divisions and forms an invaluable resource for future study of coordination between cell cycle and cell fate specification in vivo. Key words: FUCCI, cell cycle phase, embryogenesis, *C. elegans*, cell lineage

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S1-06 *C. elegans* locomotion

Andre Brown

Imperial College London, UK

Despite rapid advances in technology for sequencing and engineering genomes, it is still a challenge to associate particular genes with heritable behavioural differences because behaviour is time consuming to measure and difficult to quantify. We are using automated imaging to record the behaviour of freely moving worms and developing new analysis methods to extract relevant features. I will discuss unsupervised methods to quantify behavioural repertoires, and how making connections to language processing and data compression can give insight into the structure of behaviour. Finally I will show how these new representations can be useful for behavioural genetics.

S1-07 Gene expression of an animal germline near single cell resolution

Asija Diag[1], Marcel Schilling[1], Filippos Klironomos[2], Nikolaus Rajewsky[1, 3]

[1]*Laboratory for Systems Biology of Gene Regulatory Elements, Max Delbrück Center for Molceular Medicine*

[2]*Department of Pediatrics, Division of Oncology and Hematology, Charité*

[3]*Corresponding Author*

A key question in development is how spatial distribution of gene expression regulates cell proliferation and differentiation. A particularly important system is the animal germline where post-transcriptional regulation of gene expression is known to be fundamental for regulating proper development of germ cells through proliferation and differentiation. However, for technical reasons, the spatial resolved molecular makeup of animal germlines is largely unknown. To address this issue we are using the Caenorhabditis elegans germline as a beautiful in vivo model for proliferation and differentiation of germ cells. We used a novel cryo-cut based method (Junker et al. Cell 2014) and optimized sequencing protocols to determine, at near single cell resolution, RNA expression (mRNAs, miRNAs, siRNAs, piRNAs) as a function of position along the germline. Therefore, we capture RNA expression during the entire development of germ cells through proliferation and differentiation. Biological and technical replicates, independent in situ hybridization experiments, and mutant analyses indicate that we are able to reliably quantify the expression of the vast majority of expressed RNA molecules. Clustering of the expression data revealed that all investigated classes of RNAs are organized in groups with distinct localization patterns. We recovered localized expression patterns of known gene groups, e.g. for gld-1 targets, as well as many novel patterns, indicating new functional modules. We also discovered many new miRNAs and

3’ UTRs with germline specific expression, and an overwhelming number of siRNAs. Interestingly, some genes switch 3’UTR isoform usage along the germline, revealing new mechanistic insights in post-transcriptional regulation during germline development. Overall our data represent the first map of spatially resolved germline RNA expression and will provide crucial insights into mechanisms and function of RNA during germline development.

S1-08 Application of Imaging mass spectrometry for *C. elegans*

Yoshishige Kimura[1], Tomomi Kaneko[1], Satoka Aoyagi[2]
[1]*Department of Liberal Arts and Sciences, Kanagawa University of Human Services*
[2]*Department of Materials and Life Science, Seikei University*

Imaging mass spectrometry (IMS) is a technique that visualizes the spatial distribution of molecules and structures by their molecular masses. Images are reconstructed from the mass spectrum charts from thousands of spots from the biological tissues to show the distribution of various molecules. The commonly used ionization techniques are MALDI (Matrix assisted laser desorption ionization), DESI (Desorption Electrospray Ionization), or SIMS (Secondary Ion Mass Spectrometry). *C. elegans* is a common model organism, extensively used in life science research. Though, various investigations have been performed for metabolomic profiling of worms, the information of a single worm has been lost by the conventional mass spectrometry (MS) techniques. Thus, the development of a label-free, non-targeted MS technique for molecular mapping in *C. elegans* has been required. We have previously performed MALDI imaging of *C. elegans*. However, the resolution was not enough to analyze cellular or subcellular level of biomoleculer distribution. Thus, we next tried the application of TOF-SIMS (Time-of-Flight Secondary Mass Spectrometry) system for *C. elegans*, which enables us to obtain subcellular distribution of metabolites. We have compared several sample preparation methods and found that the frozen sections of *C. elegans* fixed by paraformaldehyde (PFA) were suitable for TOF-SIMS analysis. By sputtering of Ar gas cluster ion beam (Ar-GCIB), the sensitivity to fatty acids was significantly enhanced, and high-resolution images of biomolecules were acquired. This new imaging technique is promising to obtain the cellular and subcellular distributions of the various biomolecules, whose localization is difficult to visualize by other methods.

S1-09 Adapting a proximity labeling technique to identify novel non-centrosomal MTOC proteins in *C. elegans*

Ariana D. Sanchez[1], Tess Branon[2], Alice Y. Ting[1, 2], Jessica L. Feldman[1]
[1]*Stanford University Department of Biology*
[2]*MIT Department of Chemistry*

Microtubules have dynamically growing plus ends and comparatively stable minus ends that are nucleated, stabilized, and/or anchored at microtubule organizing centers (MTOCs). The centrosome is the primary MTOC during cell division, but in differentiated cells, MTOC function is often reassigned to non-centrosomal sites (ncMTOCs), and the composition of ncMTOCs is largely unknown. The relative simplicity and genetic tractability of *C. elegans* make it a very useful model for studying ncMTOCs. Here we are defining a more complete picture of ncMTOC protein composition by adapting a proximity labeling (PL) technique in *C. elegans*. PL offers the unique advantage of identifying proximal protein networks in vivo. One PL technique requires a promiscuous labeling BirA enzyme that covalently tags proximal proteins with biotin. Tagged proteins are then isolated and identified by mass spectrometry (MS). BirA-R118G (here referred to as BioID) emerged as a powerful PL enzyme in cell culture; however, we find that BioID is inefficient in *C. elegans*. Two variants, TurboID and miniTurbo, were engineered using directed evolution of BirA for greater biotinylation activity. We expressed BioID, TurboID, and miniTurbo in *C. elegans* differentiated epithelial cells. In the presence of biotin, TurboID and miniTurbo show significantly more biotinylated products than BioID, as detected by Western blot and immunofluorescence, suggesting that TurboID and miniTurbo are considerably more active than BioID in *C. elegans*. We have targeted TurboID to epithelial ncMTOCs by fusing it to the microtubule minus end protein PTRN-1/Patronin, which resulted in site specific biotinylation activity at the ncMTOC. We are currently optimizing biotinylation activity

conditions and streptavidin pull downs in conjunction with MS to identify novel ncMTOC components and will present our results. Overall, we have successfully adapted a PL technique in *C. elegans*, providing a powerful new biochemical tool for the discovery of novel proximal proteins in animal tissue.

S1-10 The THAP domain protein LIN-36 functions with the DREAM complex to repress transcription of proliferation promoting genes during quiescence

Csenge Gal, Alex Appert, Chiara Cerrato, Ni Huang, Julie Ahringer
Gurdon Institute, University of Cambridge

The conserved DREAM (DP, Rb-like, E2F, and MuvB) complex is a key regulator of cellular quiescence in mammals. In animals, including *C. elegans*, DREAM transcriptionally represses targets, which include many cell cycle and cell division genes, however its mode of action is not well understood. To further understanding of DREAM function, we carried out an RNAi knockdown screen for other genes needed for repression of DREAM targets, and identified the THAP domain containing protein LIN-36. Genome wide binding analyses in starved L1 larvae showed that LIN-36 extensively co-localizes with the DREAM complex at promoters. In lin-36 mutants, we observed a reduction of two tested DREAM factors; EFL-1/E2F and LIN-35/Rb at a subset of sites and the associated genes are frequently derepressed in both the lin-35 and lin-36 mutants. To investigate the role of the LIN-36 THAP domain, a putative DNA binding domain, we used CRISPR-Cas9 to delete it. In wild type, LIN-36 is found in the nuclei of most cells throughout development. In contrast, LIN-36-THAPdel has normal expression and nuclear localization during embryogenesis, but the protein is not localized to the nucleus in larvae or adults. In agreement with these findings, lin-36(THAPdel) mutants derepress DREAM/LIN-36 targets in starved L1s. Finally, lin-35 lin-36 double mutants have exacerbated phenotypes compared to either of the single mutants and show de-repression of additional target genes, indicating partial functional redundancy. The results indicate mechanistic differences in DREAM target repression: those where LIN-36 is an essential co-factor of the DREAM complex, those that require either DREAM or LIN-36 for target repression, and those that require DREAM but not LIN-36. We speculate that DREAM may have other co-factors at these latter targets.

Thursday, 14 June 2018 - Auditorium - 09:00 – 11:35

Session 2: Germline, Epigenetics & Sex Determination

S2-01 Counting to One: Patterning of Meiotic Recombination in *C. elegans*

Liangyu Zhang[1,3], Weston Stauffer[2,3], Regina Rillo[1,3], Ofer Rog[1,3,6], and Abby F. Dernburg[1,3,4,5]

[1] *Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA*

[2] *Department of Integrative Biology, University of California, Berkeley, CA, USA*

[3] *Howard Hughes Medical Institute, Chevy Chase, MD, USA*

[4] *Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley CA USA*

[5] *California Institute for Quantitative Biology (QB3), Berkeley CA USA*

[6] *Present address: Department of Biology, University of Utah, Salt Lake City, UT, USA*

A key advantage of sexual reproduction is meiotic recombination, which allows efficient purging of harmful mutations and results in new combinations of alleles. Meiotic chromosome segregation also depends on genetic exchange between homologous chromosomes, since this process creates physical links that enable homologs to separate during the first division. Although meiotic recombination is essential for meiosis, the total number of meiotic crossovers is typically very low. In *C. elegans*, as in many eukaryotes, each pair of homologs undergoes only a single crossover per meiosis, while all other double-strand breaks are repaired through non-crossover mechanisms. How information is communicated along the lengths of paired chromosomes to control crossover formation has been a longstanding mystery.

Our lab has developed methods and probes to image meiotic dynamics in living animals. This approach has enabled us to track meiotic chromosome movements (Wynne et al., 2012) and to quantify the kinetics of synapsis, the process by which a protein polymer, the synaptonemal complex (SC) assembles between homologous chromosomes. Direct observation of synapsis also led to the surprising conclusion that

the SC behaves as a liquid crystal, defined as an ordered assembly of molecules that show liquid-like mobility. Our evidence that proteins rapidly diffuse within the SC implied that this material might act as a conduit for regulatory signals along the interface between paired chromosomes.

Recent work in our lab has demonstrated that a family of meiotic RING finger proteins, ZHP-1–4, localize within the SC and regulate crossover formation. Using the auxin-inducible degradation (AID) system, we have also identified an essential role for CDK-2 in crossing-over; in this context CDK-2 interacts with the unusual cyclin COSA-1. Homologs of these proteins have been implicated in crossover control across diverse eukaryotes. Through *in vivo* imaging, biochemical analysis, and mathematical modeling, we are testing how these factors form a regulatory circuit to ensure crossover formation while limiting the number of exchanges between each chromosome pair to one.

S2-02 Tissue- and sex-specific small RNAomes reveal sex differences in response to the environment

Alexandra Bezler[1, 2], Fabian Braukmann[3, 4, 5], Sean West[6, 8], Fabio Piano[6, 7], Kristin Gunsalus[6, 7], Eric Miska[3, 4, 5], Laurent Keller[1], et al.

[1]*Department of Ecology and Evolution, University of Lausanne, Switzerland*

[2]*Present address: Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland*

[3]*Gurdon Institute, University of Cambridge, Cambridge, UK*

[4]*Department of Genetics, University of Cambridge, Cambridge, UK*

[5]*Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge, UK*

[6]*Center for Genomics & Systems Biology, Department of Biology, New York University, New York, NY*

[7]*Center for Genomics & Systems Biology, NYU Abu Dhabi, Abu Dhabi, UAE*

[8]*present address: Biologics Discovery California, Bristol-Myers Squibb, Redwood City, CA*

RNA interference related pathways are essential for germline development and fertility in metazoa and can contribute to inter- and transgenerational inheritance. In *C. elegans*, environmental double-stranded RNA can lead to heritable changes in phenotype and gene expression both through the male and female germline. Here, we use high-throughput sequencing to quantify sex-specific piRNAs, miRNAs and endogenous siRNAs in the *C. elegans* germline and the somatic gonad for the first time. We demonstrate that in contrast to the hermaphrodite germline, the male germline is resistant to RNAi triggers taken up from the environment. This tissue-specific sex-difference in silencing efficacy is associated with lower levels of RNAi amplification products. This effect is regulated specifically by germline sex, since mutant males with a feminized germline are RNAi sensitive. We thus provide mechanistic insights into sex-differences of gene regulation in response to environmental cues that may impact inheritance across generations.

S2-03 Functional characterisation of RBP-9, an RNA Pol II subunit that is required for piRNA-mediated gene silencing

Lisa Lampersberger[1, 2], Ahmet Can Berkyurek[1, 3], Eva Maria Weick[1, 3], Isabela Navarro[1, 3], Kin Man Suen[1, 3], Alper Akay[1, 3], David Jordan[1, 3], Eric Miska[1, 3, 4]

[1]*The Gurdon Institute, University of Cambridge, UK*

[2]*University of Vienna, Austria*

[3]*The Department of Genetics, University of Cambridge, UK*

[4]*Wellcome Sanger Institute, Cambridge, UK*

C. elegans and other eukaryotes have evolved genome defence pathways to distinguish self from non-self and to protect their germline from selfish genetic elements. In many animals this includes the Piwi/piRNA pathway. Some key components of the *C. elegans* Piwi/piRNA pathway are now well characterised, however, it is not understood how the Piwi/piRNA pathway triggers (co-)transcriptional gene silencing and RNA Pol II stalling. To decipher this complex pathway and its regulatory mechanisms we performed a mutagenesis screen in a piRNA sensor background to unveil additional factors. Interestingly we found a mutation in the gene coding for the RPB-9 protein, a subunit of RNA Pol II. This is the first direct evidence of RNA Pol II itself being involved in the

Piwi/piRNA pathway. Hence, we started to characterise the role of RPB-9 therein by comparing small RNA and chromatin profiles of wild-type and rpb-9 mutant animals. We found no overall changes in mature piRNA/21U-RNA profiles or their precursor transcripts, which are transcribed by RNA Pol II. This indicates that RPB-9 is not required for piRNA biogenesis but rather suggests the involvement of RNA Pol II further downstream. Interestingly, RNA Pol II ChIP-sequencing performed in wild type and rpb-9 mutant animals suggests differential binding to a set of germline-specific targets. Overall, we observe more occupancy in wild-type animals. We proposed a model of RPB-9 acting as a specificity factor for RNA Pol II, directing it to Piwi/piRNA pathway relevant gene loci. We believe our findings will provide new insights to more precisely understand the Piwi/piRNA pathway and its involvement in transcriptional regulation.

S2-04 Identification and characterization of a fertilization signal pathway for female meiosis in *C. elegans*.

Rudra Banerjee, Jens Herzog, Martin Srayko
Department of Biological Sciences, University of Alberta, Edmonton, Canada

In most sexually reproducing animals, sperm entry provides the signal to initiate the final stages of female meiosis. *C. elegans* oocytes that are activated but not fertilized, resume the cell cycle and construct a meiotic spindle. However, these cells abort meiosis during anaphase I, skip meiosis II entirely, and enter mitosis. The molecular nature of the sperm signal required for the completion of meiosis I (MI) and entry into meiosis II (MII) is still mysterious. We identified three highly similar paralogs, memi-1, 2 and 3 (meiosis-to-mitosis transition), which encode oocyte components that sense sperm entry and trigger the completion of female MI and entry into MII. Loss of all three paralogs results in a skipped-MII phenotype. In contrast, a hypermorphic mutation, memi-1(sb41ts), results in fertilized embryos that complete MI, enter MII, but are unable to exit MII properly. In a genome-wide screen for suppression of memi-1(sb41) maternal-effect lethality, we discovered gsp-3 and gsp-4, which encode highly similar sperm-specific PP1 phosphatases. Based on the suppression data, gsp-3/4 act in the same pathway as memi-1, thus, these PP1s could represent a component of the sperm signal required for completion of female meiosis. However, their direct involvement in MII has not been previously reported, possibly because complete loss of GSP-3/4 results in immotile sperm and infertility. A recent EMS screen for suppressors of memi-1(sb41) recovered 11 intragenic and 16 extragenic suppressors. Of the extragenic mutations, five were in gsp-4, consistent with our RNAi screen. In addition, 11 suppressor mutations likely represent eight new genes in the MEMI pathway. One of these encodes a putative sperm-specific protein related to glycogen synthase kinase 3 (GSK3). Pairwise combinations of different GSK3 double-mutants indicate that at least two GSK3s function redundantly. Current efforts focus on characterizing the GSK3s and assessing their role in the MEMI pathway.

S2-05 Determinants of cell fate stability in nematodes

Francesca Coraggio[1, 2], Ringo Pueschel[1, 2], Alisha Marti[1], Peter Meister[1]
[1]*Cell Fate and Nuclear Organization, Institute of Cell Biology, University of Bern, Switzerland*
[2]*Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland*

Nematodes have been widely used to characterize cell fate determination and the regulators of fate plasticity. Here we present a cell fate challenge-system based on muscle or endodermal cell fate induction, which allows tracing of successful transdifferentiation using fluorescent markers. Using this system, we show that the histone mark deposited by the Polycomb complex H3 lysine 27 methylation is dispensable for cell fate specification, but essential for the maintenance of the differentiated fate. When MES-2, the enzyme which deposits H3K27me is absent, cell fate challenge at any larval stage leads to an irreversible developmental arrest of the animals. Cell fate markers indicate that many non-terminally differentiated lineages undergo cell division when animals are challenged at the L1 stage (including M, P and V lineages). For the V lineage, this occurs even though the genome did not undergo replication, leading to arrested metaphases and a non-functional hypoderm. As the larval arrest of MES-2 animals is highly penetrant (>90% of the population), we looked for

possible regulators of cell plasticity. First and foremost, we discovered that animals in diapause or dauer stage are resistant to cell fate challenge. This resistance quickly disappears as animals feed and within half an hour, almost all animals arrest development upon cell fate challenge. This suggested that an environmental signal is sensed and transmitted mediating plasticity enhancement to exit diapause and resume development. We therefore screened a small scale RNAi library for genes whose knock-down would potentially rescue the larval arrest. Knock-down of the LIN-12Notch pathway, including ligand, co-ligands, receptor and some known target genes, rescued larval arrest as well as the cellular phenotypes. This demonstrates a role for Notch in the soma as a signal for cell plasticity enhancement upon diapause exit.

S2-06 Towards identifying genes regulating multipotency and differentiation in the SGP/hmc cell fate decision

Laura Mathies, Andrew Davies, Jill Bettinger
Virginia Commonwealth University

We use progenitors of the *C. elegans* reproductive system as a model for defining the genetic determinants of multipotency. The two somatic gonadal progenitors (SGPs) are multipotent progenitors that generate all somatic tissues of the reproductive system. Each SGP is the product of a cell division that produces one SGP and one differentiated cell, the head mesodermal cell (hmc). Therefore, in this single cell division the potential to generate all of the somatic gonadal types is differentially segregated into one daughter cell. We have used fluorescence-activated cell sorting (FACS) to isolate SGPs and hmcs from the same L1 stage worms and performed RNA sequencing to identify genes that are differentially expressed in SGPs and hmcs. We were surprised to find a large number of differentially expressed genes (~8000) when comparing these two sister cells. Importantly, we observed expression differences in several genes already known to be differentially expressed in these cells. GO term enrichment analysis of our dataset provided interesting insights. As expected, genes with higher expression in SGPs are enriched for 'reproductive system development' GO terms. Genes with higher expression in SGPs are also enriched for GO terms related to transcription and translation, as might be expected for a multipotent progenitor cell preparing to undergo multiple cell divisions. Interestingly, genes with higher expression in the hmc are enriched for GO terms related to neuronal function, such as "regulation of neurogenesis" and "synaptic vesicle exocytosis". To date, the function of the hmc has not been defined, and our data strongly suggest that it may be functioning like a neuron, an idea that is consistent with its neuron-like cellular morphology. Our ultimate goal is to identify, among these differentially expressed genes, determinants of multipotency in SGPs, as well as genes that promote the terminal differentiation of the hmcs.

S2-07 SPR-5; MET-2 maternal reprogramming antagonizes H3K36me3 in the transgenerational control of germline versus soma

Brandon Carpenter, David Katz
Emory University

Epigenetic information is acquired within each generation in order to regulate proper gene expression. In *C. elegans*, the H3K4me2 demethylase, SPR-5, and the H3K9 methyltransferase, MET-2, are maternally deposited into the oocyte and cooperate to reestablish the epigenetic ground state of the zygote by reprogramming histone methylation. Progeny of worms lacking spr-5 and met-2 exhibit a maternal effect sterile phenotype due to the rapid accumulation of H3K4me2 and the resulting ectopic expression of spermatogenesis genes in the soma. Here, we show that the progeny of spr-5; met-2 mutants display additional developmental defects including defects in gut granule accumulation, and a severe L2 developmental delay in both the germline and soma. Strikingly, these somatic defects are rescued by mes-4 RNAi. Previously, the Strome and Kelly Labs demonstrated that maternally deposited MES-4 transgenerationally maintains H3K36 at germline genes in a transcription-independent manner, and this is required to reactivate germline genes in the subsequent generation. Thus, we hypothesized that the removal of H3K4me2 by SPR-5 followed by the addition of H3K9me2 by MET-2 at fertilization may be required to prevent MES-4 from ectopically licensing germline gene

expression in somatic tissues. To test this model, we performed an RNAseq on L1 progeny from spr-5; met-2 mutants, and found that transcription-independent MES-4 targets are ectopically expressed in the soma. Thus, we propose that SPR-5; MET-2 maternal reprogramming antagonizes H3K36me3 to enable the proper transgenerational control of germline versus somatic cell fates. We are currently testing this by performing H3K36me3 ChIP-seq, and will present data from this analysis at the meeting.

S2-08 AMPK regulates germline stem cell quiescence and integrity through effects on a small RNA pathway

Pratik Kadekar, Richard Roy
Department of Biology, DBRI, McGill University, Montreal, Quebec H3A 1B1, Canada

In response to unfavourable growth conditions, *C. elegans* larvae undergo a global developmental arrest following the execution of a diapause-like state called 'dauer'. Germline stem cell (GSC) quiescence in the dauer state requires the activity of AMPK and its upstream activator LKB1 (par-4). PAR-4 and AMPK. Although wild type dauer larvae recover from this developmentally quiescent state with no negative impact on their reproductive fitness, AMPK mutant animals exhibit complete sterility after dauer exit, suggesting a loss of germ cell integrity. These animals show severe defects in germline organization and fail to make functional gametes. We were able to partially rescue this observed sterility by compromising the function of two major upstream effectors of the small interfering RNA pathway (dcr-1 and rde-4), as well as the primary Argonaute protein ergo-1. Disruption of these gene products also partially suppressed the germline hyperplasia in AMPK mutant dauer larvae. The small RNAs likely regulate gene expression by affecting histone marks deposition at the chromatin level. Consistent with this, we found that in AMPK mutant dauer larvae, the H3K4me3 and H3K9me3 chromatin marks are both increased and aberrantly distributed in the germline. The higher levels of H3K4me3 and H3K9me3 persist and fail to get resolved in the post-dauer adult germline resulting in abnormal germline gene expression. Furthermore, we show that AMPK expression in the neurons and the excretory system is sufficient to restore fertility in AMPK post dauer adults. Collectively, our data suggest that AMPK regulates a small RNA pathway, perhaps in a germline non-autonomous manner, to ensure appropriate GSC quiescence and integrity in response to the energy stress associated with the dauer stage. Our findings provide a model to understand how the soma communicates with the germ line to adapt to acute environmental challenges such as the energy stress associated with the dauer stage.

Thursday, 14 June 2018 - Auditorium - 13:00 – 15:40

Session 3: Cell biology & Early Development

S3-01 Novel facets of cell polarity in the *C. elegans* zygote

Melina Scholze, Kerstin Klinkert, Coralie Busso, Pierre Gönczy
Swiss Institute for Experimental Cancer Research (ISREC) School of Life Sciences, Swiss Federal Institute for Technology Lausanne (EPFL)

Important progress regarding the mechanisms imparting cell polarity has come from work in the *C. elegans* zygote. Following symmetry breaking, a contractile cortical actomyosin network contributes to anterior-posterior (A-P) polarity by segregating PAR proteins to discrete cortical domains. By contrast to the wealth of knowledge regarding PAR proteins and interacting components, whether specific plasma membrane components participate in polarizing the *C. elegans* zygote is poorly understood. Moreover, whereas centrosomes are thought to be essential for symmetry breaking at the onset of the polarization process, the underlying mechanisms are not clear. We will report our ongoing investigations into these two questions. First, we discovered that the plasma membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP2) forms dynamic cortical structures that are distributed in a polarized and PAR-dependent manner. We found that these PIP2 cortical structures overlap partially with F-actin, and established that F-actin is required for their formation and

movements. Reciprocally, depleting or increasing the level of PIP2 results in severe F-actin disorganization and A-P polarity defects. Overall, this work uncovers that a specific lipid plasma membrane component modulates actin organization and cell polarity in the *C. elegans* zygote. We will also discuss work in progress in which we investigate polarity establishment upon depletion of the Aurora A kinase AIR-1 or in the absence of centrosomes. Our findings lead us to propose a working model whereby the zygote possesses an intrinsic ability to break symmetry at the two poles, with the centrosomes normally ensuring that a single side is selected at the posterior of the embryo.

S3-02 A two-step mechanism for the inactivation of MTOC function at the centrosome

Jeremy Magescas, Jennifer C. Zonka, Jessica L. Feldman

Department of Biology, Stanford University, Stanford, CA, USA

During mitosis, the centrosome acts as a Microtubule Organizing Center (MTOC), forming a radial array of microtubules to segregate chromosomes between daughter cells. Microtubules are organized by the centrosome's pericentriolar material (PCM), which is steadily recruited through mitosis culminating in a peak in MTOC function in metaphase. Thereafter, PCM is removed from the centrosome, attenuating MTOC function. This inactivation of MTOC function at the centrosome is likely required for the timely exit from mitosis and for subsequent cell cycle events. While a large body of work has revealed the phosphorylation-dependent steps required for MTOC assembly at the centrosome, little is known about the mechanisms reversing this process at the end of mitosis. In *C. elegans*, the PCM is comprised of two main proteins, SPD-2/CEP192, and SPD-5, which localize the microtubule nucleating complex ɣ-TuRC. Pairwise analysis of SPD-2, SPD-5, and ɣ-TuRC revealed that the PCM proteins disassemble from the centrosome at different rates and using different behaviors. SPD-2 is removed prior to SPD-5 and ɣ-TuRC, leaving a "cage" of SPD-5/ɣ-TuRC around the centrosome. Following the gradual dissolution of SPD-2, the SPD-5/ɣ-TuRC cage ruptures into microtubule-associated "packets". The packets move towards the cortex and lose their association with ɣ-TuRC and microtubules, leaving only SPD-5 foci in the cytoplasm that eventually disappear. Using pharmacological inhibitors against serine/threonine phosphatases or let-92 RNAi, we observed a stabilization of the PCM during anaphase, implicating the PP2A phosphatase in PCM disassembly. Furthermore, increasing or decreasing cortical pulling forces using RNAi resulted in either precocious or delayed PCM removal, respectively, and altered the behavior by which proteins were removed from the centrosome. These data suggest that PCM disassembly is a two-step process, beginning with a phosphatase-dependent removal of SPD-2 followed by the rupture of the weakened PCM by cortical pulling forces, ultimately inactivating MTOC function at the centrosome.

S3-03 Aurora-A triggers local and global inhibition of contractile actomyosin networks independently of its role in centrosome maturation

Peng Zhao[1, 2], Masatoshi Nishikawa[3], Fumio Motegi[1, 2, 4]

[1]*Temasek Life-sciences Laboratory, Singapore 117604, Republic of Singapore.*

[2]*Department of Biological Sciences, National University of Singapore, Singapore 117583, Republic of Singapore.*

[3]*Hosei University, Faculty of Bioscience and Applied Chemistry, Department of Frontier Bioscience, Tokyo 184-8584, Japan.*

[4]*Mechanobiology Institute, National University of Singapore. Singapore 117411, Republic of Singapore.*

The development of multicellular organisms requires coordination of cell proliferation and cell fate diversification. Diversification of cell fate originates from asymmetric cell divisions along a polarity axis, which is usually established by spatially biased remodelling of the actin cytoskeleton. In Caenorhabditis elegans zygotes, polarization is initiated when the maturing centrosomes transiently inhibit actomyosin contractility at the posterior cortex, and is subsequently maintained when cortical actomyosin network globally disassembles. Although the dynamic remodelling of actomyosin networks has been well described, the mechanism underlying the spatio-temporal control of actomyosin networks during cell polarization remains poorly understood. Here

we show that polarization of *C. elegans* zygotes is established through sequential inhibition of actomyosin networks by the mitotic kinase, Aurora-A. Aurora-A accumulates around centrosomes to locally inhibit actomyosin contraction at the proximal cortex, resulting in asymmetric actomyosin contraction, thereby promoting cortical flows during symmetry breaking. Cytoplasmic Aurora-A subsequently mediates global disassembly of actomyosin networks, which facilitates the initial polarization through suppression of centrosome-independent cortical flows. By manipulating the spatial activity of Aurora-A, we demonstrate that Aurora-A inhibits cortical actomyosin network independently of its function in promoting centrosome maturation and cell cycle progression. We thus propose mitotic kinase Aurora-A coordinates cell polarization with cell cycle progression by acting as a diffusible cytoplasmic cue, which promotes initiation and maintenance of cell polarization through local and global modulation of contractile actomyosin networks at different stages of mitosis.

S3-04 Optogenetic control of spindle positioning

Ruben Schmidt[1, 2], Lars-Eric Fielmich[1], Anna Akhmanova[2], Sander van den Heuvel[1]
[1]*Developmental Biology Department of Biology Faculty of Sciences Utrecht University Netherlands*
[2]*Cell Biology Department of Biology Faculty of Sciences Utrecht University Netherlands*

The mitotic spindle controls the plane and orientation of cell division in animal cells. To position the spindle, cortical force generators pull on astral microtubules. A conserved complex of Gα, GPR-1/2(PINS/LGN), LIN-5(Mud/NuMA) and cytoplasmic dynein is responsible for these pulling forces, and for positioning the spindle asymmetrically. While regulators of Gα contribute to force generation, the functions of individual regulators and complex components remain poorly understood. Using a germline-specific Cre-Lox system, we observed that simultaneous loss of the Gα(GTPase) regulators RIC-8(GEF) and RGS-7(GAP) phenocopies loss of Gα itself, indicating that these regulators independently promote Gα function in force generation. To further study the role of individual force generator components, we adapted the ePDZ-LOV2 light-controlled heterodimerization system for the *C. elegans* germline. Combining membrane-bound PH::LOV and ePDZ knock-in of GPR-1/2, LIN-5, DHC-1, RIC-8, or RGS-7 we tested the functions of Gα regulation and the force generator components. This revealed that forces are restored in the absence of Gα by direct cortical recruitment of GPR-1 or LIN-5, but interestingly not dynein itself. These results indicate that Gα is not required for pulling forces, and that the LIN-5 complex does not act solely as a dynein anchor. Global recruitment of LIN-5 results in excessive and uncontrolled force generation, overruling both cell cycle and local cortical regulation, which shows that LIN-5 acts as a potent activator of dynein-dependent spindle positioning forces. By locally recruiting low levels of LIN-5 to the cortex we successfully reoriented mitotic spindles in the P0, P1, and AB blastomeres, thereby controlling the physical outcome of these early cell divisions.

S3-05 Why parthenogenetic females of Mesorhabditis belari would sire males whose DNA is not transmitted to females?

Marie Delattre[1], Manon Grosmaire[1], Pierre-Henri Gouyon[2], Marie-Anne Felix[3], Mark Blaxter[4]
[1]*LBMC/CNRS/ENS LYON*
[2]*Museum Nationale d'Histoire Naturelle Paris*
[3]*IBENS Paris*
[4]*University of Edinburgh*

Sperm-dependent parthenogenesis, also called pseudogamy, is a reproductive strategy in which females use the sperm of males, usually from another species, to activate their oocytes. The sperm DNA does not participate to the development of the zygote, which produces only females. We discovered a novel and unique reproductive strategy in the pseudogamous nematode species Mesorhabditis belari, which produces its own males. While fertilization is needed to activate all oocytes, 92% of oocytes undergo a single meiotic division, do not decondense the male DNA and develop into diploid females by gynogenesis. The remaining 8% of the oocytes undergo two rounds of meiotic divisions, the paternal DNA decondenses and mixes with the female

DNA and develop exclusively into diploid male individuals. Using game theory, we developed a model that explains why 8% of males may be maintained in these populations while their genetic material does not participate to the female fitness. Such a reproductive strategy is efficient only if sons are more likely to mate with their sisters. We experimentally tested this prediction and show that M. belari females are indeed more often fertilized, and produce more males, when mated with their brothers than with unrelated males. We sequenced the genome of M. belari and revealed a high level of heterozygosity. We are currently exploring the mechanisms that allow the maintenance of heterozygosity in the gynogenetic females. We are also exploring the sex determination system of M.belari to understand why amphimixic embryos produce only males. In parallel, we collected new Mesorhabditis strains from soil samples all around the world and identified 2 gonochoristic species and 10 new pseudogamous species that were monophyletic. Interestingly, we found a prezygotic reproductive isolation between pseudogamous species. Thus, the mating preference between siblings may be responsible for a rapid speciation within the Mesorhabditis genus.

S3-06 Patterns of contact cue diversify cell division orientation with myosin flow

Kenji Sugioka, Bruce Bowerman

Institute of Molecular Biology, University of Oregon

Cell division axes during development are specified in different orientations, thereby contributing to multicellular assembly, but the mechanisms generating division pattern diversity remain unclear. We show here that the patterns of cell contact cue can diversify the cell division orientation choice through modulating myosin flow. We reconstituted in vivo contact patterns using bead or isolated cells to show two findings. First, we identified three contact-dependent cues that pattern cell division orientation and myosin flow: physical contact, contact asymmetry, and Wnt signal. Second, we experimentally demonstrated that myosin flow generates forces to trigger plasma membrane movements and propose their anisotropy drives cell division orientation. Our data suggest that the contact-dependent controls of myosin are important to specify division axis of *C. elegans* AB, ABa, EMS cell, and mouse AB cell. The contact-dependent generation of myosin flows, in concert with known microtubule/dynein pathways, may greatly expand the division axis choice during development.

S3-07 Embryonic development in *C. elegans*: questioning the importance of yolk

Ellen Geens[1], Pieter Van de Walle[1], Liliane Schoofs[2], Liesbet Temmerman[1]

[1]Molecular and functional neurobiology, Department of Biology, University of Leuven, Naamsestraat 59 – box 2465, B-3000 Leuven, Belgium

[2]Functional genomics and proteomics, Department of Biology, University of Leuven, Naamsestraat 59, B-3000 Leuven, Belgium

Reproduction is a pivotal life-history trait as it profoundly affects the perpetuation of a species through time. Despite the fact that a multitude of reproductive strategies can be found in the animal kingdom, the clear majority of organisms are oviparous. One of the key components of an egg is nutrient-rich yolk which is produced outside the gonads and relocated to the growing oocytes during receptor-mediated endocytosis. Despite the variety in yolk composition found in animals, yolk proteins are abundantly present and serve as a carrier of lipids and carbohydrates towards the developing oocytes. Generally, it is stated that yolk is critical to drive the embryonic development of oviparous species. Previous research has already shown that yolk protein production or vitellogenesis is a well-regulated process. Recently, our research group has identified until now unknown regulators of yolk protein production or vitellogenesis in Caenorhabditis elegans, VRP-1 (vitellogenin-regulating Caenorhabditis-specific protein) and CEH-60 (*C. elegans* homeobox). Even though loss-of-function mutation of vrp-1 and ceh-60 causes yolk proteins to be absent in this nematode, the ability to produce viable offspring is not affected. This surprising observation questions the importance of yolk proteins to transport high-energy components to the developing embryos and contradicts the long-held belief of the necessity of yolk during development. Relying on differential proteomics data, assays to determine the eggs' lipid and

carbohydrate content, and conventional microscopic observations, we can determine an aberrant embryonic development of *C. elegans* in the absence of yolk and suggest a new explanation on how this organism can sustain its development under these circumstances.

S3-08 Channel Nucleoporins recruit the Polo-like kinase PLK-1 to the Nuclear Pore Complexes in prophase to direct Nuclear Envelope Breakdown in *C. elegans* embryos

Lionel Pintard[1], Lisa Martino[1], Stéphanie Morchoisne-Bolhy[1], Dhanya Cheerambathur[2], Lucie Van Hove[1], Julien Dumont[1], Nicolas Joly[1], Arshad Desai[2], Valerie Doye[1]

[1]Institut Jacques Monod, UMR7592 CNRS - Université Paris Diderot, Sorbonne Paris Cité, Paris, France

[2]Ludwig Institute for Cancer Research, Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, United States

In animal cells, nuclear envelope breakdown (NEBD) is required for the assembly of the mitotic spindle and for proper chromosome segregation. Whereas mitotic kinases have been implicated in NEBD, how they coordinate their activity in space and time to trigger NEBD is still unclear. We have shown recently that both in human cells and *C. elegans* embryos, the mitotic Polo-Like kinase 1 (PLK-1) is recruited to the nuclear pore complexes in prophase, just prior to NEBD, through its Polo-box domain (PBD). We have identified the *C. elegans* nucleoporins NPP-1/Nup58, NPP-4/Nup54 and NPP-11/Nup62, which form a trimeric complex localized in the central channel of the nuclear pore, as the critical factors anchoring PLK-1 to the Nuclear Envelope (NE). In particular, NPP-1 NPP-4 and NPP-11 primed at multiple polo-docking sites by Cdk1 and PLK-1 itself, physically interact with the PLK-1 PBD. Finally, we have provided evidence that PLK-1 localization to the NE is required for efficient NEBD. Our observations indicate that nucleoporins play an unanticipated regulatory role in NEBD, by recruiting PLK-1 to the Nuclear Envelope in prophase, thereby facilitating phosphorylation of critical downstream targets. Our objective is now to identify the PLK-1 targets at the Nuclear Envelope. Channel Nucleoporins Recruit PLK-1 to Nuclear Pore Complexes to Direct Nuclear Envelope Breakdown in *C. elegans*. Martino L, Morchoisne-Bolhy S, Cheerambathur DK, Van Hove L, Dumont J, Joly N, Desai A, Doye V, Pintard L. Dev Cell. 2017 Oct 23;43(2):157-171.e7.

S3-09 Towards understanding the importance of the unequal first cleavage of *C. elegans* embryos

Radek Jankele[1], Rob Jelier[2], Pierre Gönczy[1]

[1]Swiss Federal Institute of Technology, Lausanne (EPFL), Swiss Institute for Experimental Cancer Research (ISREC)

[2]KU Leuven, Centre of Microbial and Plant Genetics

The *C. elegans* zygote divides asymmetrically into a larger anterior AB cell and a smaller posterior P1 cell. Unequal cell volumes together with asymmetrically distributed cell-fate determinants result in a ~2 min delay in the division timing of P1 after AB. The unequal cell size and asynchrony at the two-cell stage are conserved features among Rhabditidae nematodes. Moreover, par polarity mutants that produce equally sized and synchronous two-cell stage blastomeres are lethal. Overall, unequal blastomere sizes and asynchronous timing at the two-cell stage are believed to be essential for normal embryonic development. We set out to test these postulates by producing embryos with equally sized AB and P1 that retain an asymmetric distribution of polarised fate determinants. To do so, we used embryos carrying a temperature-sensitive lin-5(ev571) allele. LIN-5 governs asymmetric mitotic spindle positioning during the first cleavage, downstream of polarity. We discovered that lin-5(ev571) results in the rapid inactivation of LIN-5 function by upshifting embryos to restrictive temperature during the first prometaphase. This results in approximately equally sized AB and P1 blastomeres. Thereafter, we followed development of these embryos at the permissive temperature by 3D timelapse microscopy to lineage them and score their elongation and hatching. We observed that lethality increases as AB and P1 become more equal, and also that all embryos with P1 larger than AB eventually die due to incomplete epidermal closure followed by expulsion of mesendodermal cells. Interestingly, we discovered that P1 descendants, but not AB descendants, exhibit accelerated cell cycle progression. Moreover, we found that cells of different origins end up in abnormal positions or divide too early, albeit in at variable

fashion. Our results to date suggest than both size asymmetry and asynchrony are necessary for robust embryonic development of *C. elegans*.

Thursday, 14 June 2018 - Auditorium - 16:05 – 18:35

Session 4: Cell Fate I (postembryonic)

S4-01 Orienting cell polarity by Wnt signaling

Hitoshi Sawa

Multicellular Organization Laboratory, National Institute of Genetics, Mishima, Japan

During development, cells in a tissue are often polarized in the same orientation. Such coordinated cell polarity is essential for proper patterning, morphogenesis and functions of tissues. Although it is well know that polarity of migrating cells is instructed by gradients of chemokines, it is poorly understood how polarity orientation of non-migrating cells is regulated by extrinsic signals. In most animals, Wnt signaling is important for cell polarity regulation. However, the functions of Wnts as global polarity cues have not been clearly demonstrated in any organisms. In *C. elegans*, polarity of most cells undergoing asymmetric divisions is regulated by Wnt signaling, resulting in asymmetric nuclear localization of POP-1/TCF (higher in the anterior than the posterior daughters). We showed that proper polarity orientation of seam cells requires Wnts. However, Wnts appear to function permissively, since ectopically expressed Wnts can properly regulate the polarity (Yamamoto at al. 2011). To the contrary, we recently found that such ectopic Wnts instruct polarity orientation in mutants of lin-17/Frizzled. These results suggest that Wnts have both instructive and permissive functions in orienting cell polarity. In contrast to seam and most other cells, the Z1 cell, one of somatic gonadal precursors (SGPs) has opposite polarity orientation. We found that polarity of SGPs is redundantly regulated by Wnts and LIN-17. In a compound strain with wnt and lin-17 mutations, we found strong correlation between polarity of Z1 daughters and migratory direction of DTC derived from Z1 (a granddaughter of Z1), suggesting that migration of DTC is controlled by polarity of its mother cell.

S4-02 In vivo reprogramming of coelomocytes

Anna Reid[1], Ismail Özcan[1], Margaux Quiniou[1, 2], Andreas Ofenbauer[1], Baris Tursun[1]

[1]*Berlin Institute for Medical Systems Biology (BIMSB) at the Max Delbrück Center (MDC) for Molecular Medicine in the Helmholtz Association, Berlin, Germany*

[2]*University of Exeter, Exeter, UK*

Understanding how cells maintain and safeguard their fate in vivo is a crucial step towards direct reprogramming of differentiated cells into other cell types. In *C. elegans*, cellular conversion events induced by natural transdifferentiation and ectopically expressed transcription factors (TFs), provide a simple model to investigate reprogramming in an in vivo setting. To understand the mechanisms of how cellular context influences a cell's ability to convert its fate and how cellular identities are shut down while the target fate is being established, we are making use of our newly discovered TF-induced transdifferentiation event. We found that mesodermal coelomocytes (CCs), which have scavenging and hepatic functions, can be converted to intestinal-like cells upon overexpression of the GATA TF ELT-2. Remarkably, CCs can also be converted to a more distant lineage by the zinc finger TF CHE-1, which specifies the fate of gustatory ASE neurons. Ectopic CHE-1 expression reprograms differentiated CCs, which acquire neuron-like cell morphologies, express neuron-specific reporters and immunostain for the synaptic protein UNC-10 (Rim1 homolog). Importantly, a portion of CCs show growth of neuron-like projections coupled with strong repression of the CC fate, however, approximately 40% of animals do not show CC conversion, suggesting that there are barriers in place to prevent reprogramming. To tease apart the mechanisms behind CC conversion into neuron-like cells, we knocked down a set of genes involved in chromatin regulation and have identified a number of putative enhancers and suppressors of reprogramming. Additionally, we are applying single-cell RNA and ATAC sequencing to the

conversion of CCs into neuron-like cells to identify transcriptomic changes and chromatin dynamics during reprogramming in vivo. Using our novel system, we aim to gain insight into the mechanisms by which cells are amenable to TF-mediated reprogramming and how the original cell fate is suppressed.

S4-03 A natural transdifferentiation event involving mitosis is empowered by integrating signaling inputs with conserved plasticity factors

Claudia Riva, Christelle Gally, Martina Hadjuskova, Sophie Jarriault

In vivo cellular plasticity and direct reprogramming, IGBMC, University of Strasbourg, CNRS UMR7104, INSERM U1258

Transdifferentiation is the direct conversion of one differentiated cell type into another, with or without cell division. Natural transdifferentiation was characterized in *C. elegans* by our lab, which demonstrated that the Y rectal cell transdifferentiates into the PDA motor neuron with high efficiency, robustness and irreversibly (Jarriault et al., 2008). Pluripotency-associated factors, chromatin modifiers and developmental genes involved in Y-to-PDA Td were identified (Richard et al., 2011; Kagias et al., 2012; Zuryn et al., 2014). We examined if other plasticity events occur in the worm checking the somatic cell lineage (Sulston et al., 1983) to define what core mechanisms, if any, and what event-specific variations can be unraveled. We focused on: (1) Y-to-PDA in males which appears to happen in a sex-specific manner; (2) the formation of the DVB neuron from the K rectal cell, another putative transdifferentiation event; (3) transdifferentiation that could underlie the formation of the two RMH neurons from the G1 pore cell. Since all these events involve a cell division, we explored the role of transdifferentiation factors, the impact of the division, and their relationship. Expression of marker genes and cellular morphology confirmed that initial and final identities are completely different, suggesting a bona fide transdifferentiation in all these cases. Our data point to the existence of a "plasticity cassette", important for all transdifferentiation events, and event-specific factors. Focusing on K, the activity of plasticity cassette genes, which act downstream of cell division, is crucial to allow K.p daughter to erase its epithelial identity, while the orientation of the division together with Wnt signaling is needed to activate the expression of genes like terminal selectors necessary for DVB identity. Thus, in this event, two parallel and necessary processes are at play, one to erase the initial identity and the other to subsequently superimpose the final one.

S4-04 Cross-talk between the NOTCH and hypoxia response pathways modulates RAS/MAPK-mediated cell fate decisions in *C. elegans*

Sabrina Maxeiner[1, 2], Judith Grolleman[3, 4], Tobias Schmid[1], Jan Kammenga[3], Alex Hajnal[1]

[1]*University of Zurich, Institute of Molecular Life Sciences, Winterthurerstrasse 190, Zurich CH-8057, Switzerland*

[2]*PhD Program in Molecular Life Sciences, University and ETH Zurich, Switzerland*

[3]*Laboratory of Nematology, Wageningen University, Droevendaalsesteeg 1, 6708PB, Wageningen, The Netherlands*

[4]*Radboud University Nijmegen Medical Centre, Department of Human Genetics, Nijmegen, The Netherlands*

Oxygen homeostasis is crucial during normal animal development and frequently imbalanced during tumorigenesis. Under hypoxia, animals reduce their metabolic activity and activate the hypoxia-inducible factor HIF to adapt many cellular functions, such as increasing VEGF signalling to induce angiogenesis. However, it is not known how the related EGFR/RAS/MAPK pathway responds to changes in oxygen concentrations. A direct link between RAS/MAPK and hypoxic signalling has recently emerged in *C. elegans* through the identification of two polymorphic genes modifying RAS/MAPK signaling. RNAi knock-down of a calpain homolog and the VHL binding protein 1 not only suppressed the egg-laying-defective phenotype of HIF-1 prolyl hydroxylase (egl-9) mutants, but also affected RAS/MAPK-induced cell fate specification. We have further investigated the cross-talk between the hypoxia and RAS/MAPK signaling pathways in different tissues of the hermaphrodite. Hypoxic treatment of let-60(gf) animals reduces RAS/MAPK signalling during duct fate specification, pachytene exit of germ cells and vulval development. Moreover, a hif-1(lf) mutation is epistatic to an egl-9(lf) allele that suppresses increased RAS/MAPK signalling in the vulval precursor cells (VPCs) under normoxia. Thus, the hypoxia-response pathway counteracts RAS/MAPK activity via HIF-1 even under normoxia. By screening the

known HIF-1 transcriptional target genes we identified the nuclear hormone receptor NHR-57 as a critical inhibitor of RAS/MAPK signalling. Furthermore, lateral NOTCH signalling induces EGL-9 expression to promote HIF-1 degradation in uncommitted VPCs. This creates the following positive feedback loop promoting vulval fate specification: At the onset of vulval differentiation, RAS/MAPK-induced expression of DELTA family NOTCH ligands activates egl-9 expression in the proximal VPCs, which represses HIF-1 and NHR-57 expression and keeps the VPCs competent to differentiate. This regulatory network formed by the NOTCH, hypoxia and RAS/MAPK pathways permits adaptation of developmental processes to variations in oxygen concentration.

S4-05 Roles of microRNAs in embryonic development

Luisa Cochella

IMP Vienna

MicroRNAs (miRNAs) are short, non-coding RNAs that direct post-transcriptional repression of gene expression in a sequence-dependent manner. They are essential for regulation of gene expression in multicellular animals and plants, as evidenced by embryogenesis defects in individuals lacking the enzymes that process miRNAs. However, loss of most individual miRNAs has little or no effect on embryo development, and almost two decades after their discovery, the functions of most miRNAs remain unknown. Recent work from my lab has allowed to propose the following model for how miRNAs contribute to embryogenesis in *C. elegans*: a few, redundantly acting miRNAs account for the essential and early requirement of the miRNA biogenesis enzymes, while the majority of miRNAs is expressed in highly restricted cell populations and likely play roles at later stages in the differentiation and/or function of specific cell types.

S4-06 Partial versus complete loss of SWI/SNF function leads to opposite cell division phenotypes

Molly Godfrey, Aniek van der Vaart, Vincent Portegijs, Sander van den Heuvel

Developmental Biology, Utrecht University

Combining cell-division arrest with terminal differentiation is critical for normal development and tumour suppression, yet it remains poorly understood how these processes are jointly regulated. Previously, we showed that the SWI/SNF chromatin remodeling complex acts redundantly with cell cycle inhibitors to promote cell cycle exit during differentiation in the mesoblast lineage (Ruijtenberg and van den Heuvel, Cell 2015). Incomplete loss-of-function of SWI/SNF core subunits resulted in extra divisions of muscle precursor cells, and when combined with loss of G1/S regulators created a tumorous phenotype. More recently, we have found that the BAF (rather than PBAF) version of the SWI/SNF complex is predominantly involved in the control of cell-cycle exit during differentiation. However, lineage-specific knockout of endogenous BAF genes produced a surprising phenotype, with hyperproliferation of muscle precursor cells in early post-embryonic development, while later-formed precursors prematurely arrest cell division. Further experiments revealed that these opposite effects depend on levels of residual SWI/SNF activity. Incomplete inactivation of SWI/SNF core subunits or the BAF-specific SWSN-8(ARID1) interferes with proper arrest of cell division, while gene knockout combined with targeted protein degradation causes acute cell division arrest. This strong dosage dependency supports that SWI/SNF-mediated remodelling promotes cell cycle arrest, while its complete absence is incompatible with proliferation. Suppressor analysis indicates that the essential function of the complex does not reside in the known activity of SWI/SNF as Polycomb repressor antagonist. We are currently performing single molecule mRNA FISH and tissue- and developmental-stage-specific transcriptome analysis experiments, in order to reveal the essential functions of SWI/SNF chromatin remodelling complexes in promoting proliferation as well as their contribution to cell division arrest.

S4-07 The evolutionarily conserved DEAD-box helicase DDX-23 functions in stem cell biology

Akiko Doi[1, 2], Bob Horvitz[1, 2]

[1]*HHMI*

[2]*Dept. Biology, MIT, Cambridge, MA 02139 USA*

During animal development, gene regulation needs to be temporally precise for proper cell-fate decisions to occur. The evolutionarily conserved *C. elegans* heterochronic pathway controls the temporal progression of development by regulating the activities of a sequence of genes. Components of this pathway control cell-fate decisions of proliferation versus differentiation, and mammalian homologs of these components play critical roles in stem cell regulation and cancer biology. *mab-10* encodes the *C. elegans* NGFI-A-binding protein (NAB) transcriptional co-factor. MAB-10 is involved in the terminal differentiation of the hypodermal stem-like seam cells and more generally in the larval-to-adult transition. LIN-29, the master regulator of the larval-to-adult transition, is an early growth response (EGR) protein that acts together with MAB-10 to control the expression of genes that regulate the onset of adulthood and terminal differentiation in the hypoderm. Strikingly, EGR proteins interact with NAB proteins to cause terminal differentiation and the onset of puberty in mammals. Despite the importance of this pathway, mechanisms by which the terminal effectors LIN-29(EGR) and MAB-10(NAB) function remain largely unknown. We are studying LIN-29(EGR) and MAB-10(NAB) with the goal of understanding the mechanisms that control *C. elegans* developmental timing and providing insights concerning stem cell identity and development in mammals. We performed genetic screens for enhancers of the *mab-10* mutant phenotype and identified the DEAD-box helicase gene *ddx-23* as a regulator of seam cell fate. We showed that DDX-23 functions in the seam cells to regulate seam cell exit from the cell cycle. Our data suggest that DDX-23 and MAB-10 act in a complex to regulate the decision for the seam cells to terminally differentiate. We propose that this interaction is evolutionarily conserved and act to regulate mammalian stem cell development. We hope to elucidate the exact mechanism by which DDX-23 and MAB-10 function in stem cell biology.

Thursday, 14 June 2018 – Port Vell Room - 18:35 – 21:05

Poster session I

P-01 Maternal age generates phenotypic variation among genetically identical individuals in C. elegans

Marcos Francisco Perez[1, 2], Mirko Francesconi[1, 2], Ben Lehner[1, 2, 3]

[1]EMBL-CRG Systems Biology Research Unit, Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Dr. Aiguader 88, Barcelona 08003, Spain.

[2]Universitat Pompeu Fabra (UPF), Barcelona, Spain.

[3]Institució Catalana de Recerca i Estudis Avançats (ICREA), Pg. Lluis Companys 23, Barcelona 08010, Spain.

For unknown reasons, genetically identical animals often differ substantially in their phenotypic traits, even in a controlled environment. We investigated the causes of inter-individual physiological variation in Caenorhabditis elegans populations. Isogenic nematodes vary in their size at hatching, speed of development, growth rate, starvation resistance and fecundity. We show that much of this variation is due to the age of an individual's mother, with young mothers producing progeny impaired for many traits. The underlying molecular mechanism for multiple traits is a progressive, age-dependent increase in the maternal provisioning of a lipoprotein complex, yolk/vitellogenin, to embryos. The production of sub-optimal progeny by young mothers may reflect a trade-off between the competing fitness traits of a short generation time and progeny survival and fecundity. These results identify age-dependent changes in maternal provisioning to embryos as an important source of phenotypic variation throughout the life of an animal. We will present our latest results on the impacts of maternal age on offspring and on the mechanisms by which maternal age, and embryonic vitellogenin levels, act on organismal physiology. References Perez, M. F., Francesconi, M., Hidalgo-Carcedo, C. & Lehner, B. Maternal age generates phenotypic variation in Caenorhabditis elegans. Nature 552, 106-109 (2017).

P-03 Ribosomal RNA methylation by rram-1 modulates healthy lifespan and development

Markus Schosserer[1, 2], Jarod A. Rollins[2], Clemens Heissenberger[1], Fabian Nagelreiter[1], Santina Snow[2], Aric Rogers[2], Johannes Grillari[1]

[1]*University of Natural Resources and Life Sciences, Vienna / Department of Biotechnology*

[2]*Mount Desert Island Biological Laboratory*

The ribosome has been seen for decades as a static machine that translates mRNAs into proteins. However, over the last few years it became clear that it rather represents a highly dynamic structure that responds to various stimuli by adapting its structure and, as a consequence, its function. Such structurally distinct ribosomes are postulated to be "specialized ribosomes" comprising peculiar functional properties and are thus considered to be engaged in translating specific subsets of cellular messages (Filipovska and Rackham, 2013; Xue and Barna, 2012). Although ribosomal RNA is extensively modified by methylations and pseudouridinylations (Rozenski et al., 1999), the functional roles of such modifications in regulating translation are not understood. m1A674 methylation of 26S rRNA is introduced by rram-1 in Caenorhabditis elegans (Yokoyama et al., 2018), is preferentially associated with fully assembled 80S ribosomes and modulates translation of a specific set of mRNAs. Interestingly, both rram-1 expression and methylation at A674 are influenced by age and feeding protocol. Furthermore, knockdown of rram-1 extends lifespan and improves locomotion at advanced age, but severely delays development. Thus, methylation of ribosomal RNA represents an important regulator of organismal aging and our work will contribute to a better understanding of the precise molecular mechanisms underlying this healthspan modulation.

P-05 A novel set of non-coding RNAs have critical roles in nematode spliced leader trans-splicing

Rotimi Fasimoye, Bernadette Connolly, Berndt Müller, Jonathan Pettitt

Institute of Medical Science, School of Medicine, Medical Science and Nutrition, University of Aberdeen, AB25 2ZD Aberdeen, Scotland, United Kingdom.

In spliced leader (SL) trans-splicing, the 5' end of an mRNA is donated by a separate RNA called the SL RNA in a process related to cis-splicing. The SL RNA is packaged in ribonucleoprotein (RNP) complexes which, as the SL RNA is used up during trans-splicing, need to be continuously replaced. Important SL trans-splicing specific factors in C. elegans such as SNA-2, SNA-1 and SUT-1 have been identified. Loss-of-function of sna-2 gene results in larval lethality while loss of function of either sna-1 or sut-1 caused less severe phenotype. However, compound loss-of-function of sna-1 and sut-1 is lethal, an indication that they genetically interact.(MacMorris et al. 2007;Denker et al. 2002;Philippe et al 2017). SNA-1 and SNA-2 are part of the SL1 RNP complex in C. elegans. SUT-1, a paralog of SNA-1, interacts with a set of enigmatic RNA species called SmY RNA which form another SNA-2-containing RNP complex. SmY RNAs, are structurally related to SL RNA although they are unrelated at the primary sequence level beyond the ability to bind Sm proteins. There are 12 SmY RNA genes in C. elegans, making genetic analysis challenging. We have created loss-of-function mutations in 10 smy genes(smydel-10) using a CRISPR/Cas9 technique and observed the inhibition of SL trans-splicing in the strains carrying these mutations. Furthermore, loss of function of sna-1 in parallel to the smydel-10 mutations results in larval lethality. Thus, we have shown that SmY RNAs have a critical role in SL trans-splicing. However the precise role of the SmY RNA is not yet understood. Future work includes deletion of the remaining two smy genes, for a full analysis of smy function. Furthermore, we intend to analyse the level of Sm proteins in smy mutants to test the hypothesis that these complexes act in part to recycle Sm proteins following trans-splicing reactions.

P-07 C. elegans Natural Diversity Resource

Robyn Tanny, Erik Andersen

Northwestern University

Caenorhabditis elegans is used as a model in almost 1,300 labs around the world and has enabled biological discoveries in many diverse fields. These advances have been fueled by an extensive genetic toolkit including strains, reagents, and databases. However, the majority of C. elegans research focuses on the laboratory-domesticated N2 strain, neglecting potential insights gleaned from natural populations. Studies of C. elegans natural variation can identify the genetic factors underlying biomedically relevant traits and genome evolution. A variety of research groups have also used natural variation to study a multitude of other important traits including Ras pathway signaling, dauer formation,

behaviors, and longevity. To conduct studies of natural variation in C. elegans, a single laboratory needs expertise in diverse areas such as statistics computational skills, whole-genome sequencing, and quantitative phenotyping. To address the needs for these groups, we developed the Caenorhabditis elegans Natural Diversity Resource (CeNDR) - available at www.elegansvariation.org. The web-based CeNDR platform includes three areas: (1) a central repository for the deposition, organization, and distribution of wild C. elegans strains including detailed information for each strain (e.g. collection date, GPS location, substrate, and elevation); (2) a data portal for dissemination of whole-genome sequence data in BAM or CRAM formats and variant data in VCF format for each of the 249 wild isolates, including a powerful interactive genome browser that can be used to interrogate genetic variation across the population for genes or regions of interest; (3) a genome-wide association mapping portal to enable mappings of quantitative traits measured using wild C. elegans strains, including a comprehensive report of significance, variation, measures of selection, etc. We believe that CeNDR will become an indispensable tool within the C. elegans genetic toolkit to enable researchers to examine natural populations and identify interesting new biological phenomena.

P-09 Satellites in nematodes: a standing puzzle

Juan A. Subirana[1, 2], M. Mar Albà[2], Xavier Messeguer[1]

[1]Department of Computer Science Universitat Politècnica de Catalunya Barcelona, Spain

[2]Research Programme on Biomedical Informatics (GRIB) Hospital del Mar Research Institute(IMIM) Universitat Pompeu Fabra (UPF) Barcelona, Spain

Holocentric nematodes have a large number of satellites, defined as tandem repeats of a motif longer than ten bases. We have analyzed their distribution in the genome and found that they are unrelated in different species. About half of the satellites are unique, whereas the other half have been transposed over several chromosomes and form families with the same repeated motif. The X chromosome shows special features: we have found satellite families unique for this chromosome, whereas several widespread families are only found in the autosomes. Comparison of the related species C. briggsae and C. nigoni shows that satellites are shorter and less abundant in the hermaphrodite species C. briggsae: satellite transposition and growth show a higher activity in the unisexual species C. nigoni. Transposition appears to be favored by the presence of conserved adjacent sequences at both ends of the satellites. The eventual role of satellites in the genome is unclear, but we have found that long satellites and satellites with longer repeat motifs are found in regions with a higher affinity for CENP-A, a centromere specific histone. This suggests that some satellites may play a role in mitosis. We have also compared the results obtained from different assemblies recently obtained with new sequencing methods for the genome of C. elegans . We conclude that about 20 % of the satellites described in the standard ce11 assembly are too short, missing several repeats.

P-13 Sumoylation regulates protein dynamics during meiotic chromosome segregation

Ronald Hay, Federico Pelisch

Centre for Gene Regulation & Expression, School of Life Sciences, University of Dundee

In C. elegans meiosis, two-step cohesion loss is achieved by the creation of two functionally distinct domains separated by the crossover (CO). Because the CO has an off-center position, these domains differ in length and are termed short arm, which loses cohesion in meiosis I, and long arm, which loses cohesion in meiosis II. Before segregation, the short arm is enriched in the kinase Aurora B in a Haspin- and CDK1-dependent manner, leading to the release of cohesion. Conversely, the long arm becomes enriched in Protein Phosphatase 1 (PP1), antagonising Aurora B, therefore maintaining sister cohesion during the first division. In addition to Aurora B, the short arm is a hotspot for conserved cell division regulators like Bub1, Mad1, CLASP, Kif4A, and Separase. This set of proteins displays a ring-shaped localisation pattern during metaphase and all the components exhibit an extremely dynamic localisation pattern during chromosome segregation. Remarkably however, the functional relevance and regulatory mechanisms of this dynamic behaviour remain unclear. We have recently uncovered that assembly of the short arm protein network depends on the small ubiquitin-related modifier SUMO, which acts through a combination of covalent and non-covalent interactions (SUMO network). We also found that SUMO conjugation is highly dynamic during meiosis, being tightly regulated by a SUMO E3 ligase and SUMO proteases. In fact, while SUMO conjugation is required for congression and segregation, desumoylation is required specifically for chromosome segregation. We have found that

two SUMO proteases, ULP-1 and ULP-4 regulate SUMO chains in vivo and knockdown of ULP-1 and ULP-4 leads to altered protein localisation dynamics. These results provide new insights into the regulation of meiotic chromosome segregation, and also offer a great model to address the long-standing question of how the balance between SUMO E3 ligases and SUMO proteases is achieved in vivo.

P-15 Systematic analysis of atx-2 suppressors reveals a novel regulator of PAR-5/14-3-3sigma function during mitosis in Caenorhabditis elegans.

Megan Gnazzo, Alex Villarreal, Ahna Skop
UW-Madison

RNA regulation plays a critical role in mitosis, but the precise molecular mechanisms are unclear. Our recent genetic analysis in C. elegans demonstrated that the conserved RNA-Binding Protein (RBP), ATX-2, regulates cytokinesis by targeting ZEN-4 to the spindle midzone, acting through the conserved translation regulator PAR-5/14-3-3sigma (Gnazzo et al., 2016). Co-depletion of ATX-2 and PAR-5 restored ZEN-4 targeting to the spindle midzone, but did not rescue cell division. Here we extend those results by conducting a two-part candidate RNAi suppressor screen, identifying additional ATX-2-interacting factors that are 1) important for cell division, and 2) mediate ATX-2 targeting to the spindle midzone. We thus identified ten genes that, when depleted, suppress the embryonic lethality observed in atx-2 mutant embryos. Additionally, five of these genes (cgh-1, cki-1, vab-3, vhl-1, vps-24) are required for normal targeting of ATX-2 to the centrosomes and midzone. The strongest suppressor of the atx-2 phenotype is CGH-1/DDX6, a DEAD-box RNA helicase implicated in RNA processing, translation, and neuronal function. Loss of CGH-1 rescued the cytokinesis defect in atx-2 mutants, and restored normal ZEN-4 targeting to the spindle midzone. We conclude that ATX-2 and CGH-1 are mutually required for proper localization during mitosis, and coordinately regulate normal cytokinesis. Our findings provide the first functional evidence that CGH-1/DDX6 regulates ATX-2 function during mitosis to target ZEN-4 to the spindle midzone via PAR-5/14-3-3sigma, and may provide mechanistic insight into ATXN-2-mediated human diseases such as SCA2, ALS, and neurodegeneration.

P-17 Connecting social behaviour and phenotypic plasticity through the nekl-4 kinase in Pristionchus pacificus nematodes

Eduardo Moreno, Maša Lenuzzi, Christian Rödelsperger, Hanh Witte, Ralf Sommer
Max Planck Institute for Developmental Biology, Max-Planck-Ring 9, 72076 Tuebingen

Nematodes respond to a multitude of environmental cues, which are integrated into the neural circuit through sensory neurons and produce two kinds of responses: adaptive behaviours and developmental decisions that influence morphological plastic traits. The beetle-associated nematode Pristionchus pacificus performs oxygen-induced social behaviours, similarly to Caenorhabditis elegans, which are regulated by sensory cilia in both species. Mutations affecting intraflagellar transport (IFT) components induce social behaviours in both C. elegans N2 and P. pacificus PS312 wild type animals. Contrary to C. elegans, P. pacificus shows phenotypic plasticity in the feeding structures, resulting in two distinct morphs adapted to feed on different food sources. Specifically, eurystomatous morphs have two movable teeth and are facultative predators on other nematodes, whereas stenostomatous morphs have only one tooth and feed exclusively on bacteria. Recently, we have discovered a mouth-form phenotype associated with mutations in some of the IFT components. We have studied further the combined regulation of social behaviours and mouth-form dimorphism by means of a suppressor screen performed on a klp-20;osm-3 double kinesin mutant. This experiment allowed us to identify the kinase nekl-4 as an important component of the signal transduction pathway that integrates environmental inputs into the neural circuit. Given the lack of information about the function of nekl-4 in nematodes, we are currently performing a functional characterizing of nekl-4 in P. pacificus, by analysing its expression pattern and the epistatic interactions with previously described mouth-form regulatory genes. Besides, we are analysing the phosphoproteome of nekl-4 mutants in order to identify its interacting partners.

P-19 Post-mitotic roles of MEL-28 in gene expression and lifespan regulation

Raquel Romero Bueno[1], Celia María Muñoz Jiménez[1], Peter Askjaer[1], Agnieszka Dobrzynska[1], Georgina Gómez Saldivar[2]

[1]*CABD/UPO/CISC*

[2]*Department of Biology/ UNIFR*

Nucleoporins are the constituents of nuclear pore complexes (NPCs) and are essential regulators of nucleocytoplasmic transport, gene expression and genome stability. The nucleoporin MEL-28/ELYS plays a critical role in NPC assembly through recruitment of the NUP107-160 subcomplex, and is required for correct meiotic and mitotic chromosomes segregation. However, MEL-28 is also expressed in post-mitotic cells, suggesting that it might have additional functions. In support of this, we have observed that mel-28 mutants have a dramatically reduced lifespan, both in the presence of proliferating germ cells and in sterile glp-4 animals. We have mapped several functional domains in MEL-28, including a C-terminal DNA binding domain. Combined with the observation that a significant fraction of MEL-28 localises in the nucleoplasm, we speculate that MEL-28 might be directly involved in control of gene expression. To identify genes potentially regulated by MEL-28 we performed DamID experiments. This revealed that MEL-28's binding profile is different from those of others nuclear envelope proteins (NPP-22/NDC1, LMN-1/lamin and EMR-1/emerin), associating more frequently with chromosome centres. Interestingly, we found a positive correlation between MEL-28 peaks and active transcription markers, such as AMA-1/RNA pol II and methylated histone H3K4 and H3K36. In contrast, LMN-1 and EMR-1 are enriched outside MEL-28 associated domains (MADs). Moreover, expression levels of genes in MADs are higher than in MEL-28 gaps. MADs are enriched for genes involved in general cell biology processes but also larval development and locomotion. To analyse the functional relevance of MEL-28's association to chromatin, we are currently studying the effect of MEL-28 depletion on nuclear organization and gene expression. Finally, we will report behavioural data of mel-28 mutants, including developmental progression using a novel luciferase-based high-throughput method.

P-21 Pattern formation by dancing

Daniel Findeis, Christian Hennig, Ralf Schnabel

Institute of Genetics, Technical University Braunschweig, Braunschweig, Germany

Schnabel and co-workers proposed that pattern formation in the C. elegans embryo is the result of a sorting process involving extensive cell migrations. These migrations establish stereotypic regions originating from the somatic founder cells. This process, termed "cell focussing", is governed by local cell-cell interactions, where "cell addresses" — which are part of their identities — are matched. When cell identities are altered (in a glp-1 (Notch) embryo, for example), cells sort in a spectacular, new pattern corresponding to their new identities (Schnabel et al., Dev. Biol, 2006; Bischoff and Schnabel, Dev. Biol 2006). It appears to be the conventional wisdom that cells remain stationary and initiate migration only upon a specific signal. In contrast to this we observe that cells in the C. elegans embryo are constantly, independently and randomly moving back and forth at least until the onset of morphogenesis – a process we termed "dance" of cells. We now showed that the loss of the activity of components of the CED-10/Rac-WAVE-Arp2/3 pathway, known to regulate actin dynamics, results in a significant reduction of effective migration towards the terminal positions at the end of the premorphogenetic stage. In the strong maternal effect wve-1 (ok3308) mutant, guided migrations are essentially eliminated since cells are just dancing around the positions reached by mitoses only. The general dance movement remains — quite enigmatically —unchanged. Finally, we propose that the CED-10 pathway defines the link of the random dance to the cell addresses guiding the sorting of cells to their final positions.

P-23 Mixing of parental genomes after fertilization in C. elegans involves a stepwise pronuclear membrane fusion process that connects the two pronuclei through multiple fenestrations

Orna Cohen-Fix[1], Mohammad Rahman[1], Adam Harned[2], Irene Chang[2], Kedar Narayan[2]

[1]*The Laboratory of Cell and Molecular Biology, NIDDK, NIH*

[2]*Center for Molecular Microscopy, Frederick National Laboratories for Cancer Research*

In a fertilized embryo, the nuclear envelopes (NEs) of the maternal and paternal pronuclei must break down to allow mixing of the two parental genomes. To date, the main focus has been on the fate of NE proteins in this process. In C.

elegans, these dissociate from the NE after pronuclear meeting, leaving behind the double membranes of each pronucleus. The process that breaches these membranes is unknown. To address this, we followed nuclear membrane architecture in one-cell embryos from prometaphase to anaphase using Focused Ion Beam - Scanning Electron Microscopy (FIB-SEM) at nanoscale resolution. After pronuclear meeting, the maternal and paternal chromosomes are separated by four nuclear membranes. We found that in prometaphase, the two pronuclei become linked through membrane junctions involving the outer nuclear membranes of both pronuclei, generating 4-way junctions. This is followed by a reduction in the number of membranes between the two pronuclei from four to two, possibly by movement of membrane material away from the pronuclear interface through these junctions. As the cell reaches metaphase, the entire interface between the two pronuclei is composed of only two nuclear membranes. These form a flat surface surrounded by three way junctions, wherein four pronuclear membranes are reduced to two. This flat surface is fenestrated by multiple holes ranging from tens of nanometers to several microns in diameter. At metaphase, the chromosomes of the two pronuclei mix through one of the larger fenestrations, which then expands to take up most of the interface. At anaphase the membrane interface disappears. Thus, in C. elegans, the two pronuclei do, in fact, fuse. Pronuclear membrane junctions and the multiple fenestrations were not previously detected in any system. Moreover, the presence of membrane junctions suggests that parental genome mixing requires a membrane fusion machinery, the nature of which is under investigation.

P-25 Tissue-specific repair activity of ERCC1/XPF in C. elegans

Mariangela Sabatella, Karen Thijssen, Wim Vermeulen, Hannes Lans
dpt. Molecular Genetics, Erasmus MC

Defects in the DNA Damage Response (DDR) affect tissues differently, suggesting that genome maintenance operates in a tissue-specific manner. A prime example is given by hereditary defects in ERCC1/XPF, which can give rise to different diseases characterized by a complex heterogeneity of clinical symptoms. ERCC1/XPF is a structure specific endonuclease that is critical for DNA incision during Nucleotide Excision Repair (NER) and other DNA repair mechanisms. NER consists of two sub-pathways: Global Genome NER (GG-NER), which deals with damage anywhere in the genome, and Transcription-Coupled NER (TC-NER), which deals with damage that blocks transcription. The function of ERCC1/XPF in NER has been studied in great detail but it is still not clear how defects in the activity of this complex can lead to a variety of tissue-specific symptoms. In C. elegans, ERCC-1/XPF-1 deficiency causes developmental defects and accelerated replicative aging, reminiscent of symptoms in human patients. To better understand to what extent DNA repair functions differently in tissues, we monitored spatio-temporal dynamics of fluorescently tagged ERCC-1/XPF-1 in different tissues, including the germline, neurons and muscles, in unperturbed conditions and in response to DNA damage. Strikingly, ERCC1/XPF exhibits tissue-specific activity. In germ cells, the endonuclease complex quickly but transiently re-localizes to and binds UV-damaged chromosomes in a GG-NER-dependent manner, but this DNA damage response changes upon differentiation of cells. Our results confirm that in vivo the main role of GG-NER is to safeguard the whole genome in the totipotent germline, while the main role of TC-NER seems to safeguard active genes to promote cell function in differentiated tissues.

P-27 Study of the fate of cellulose in *C. elegans*, its impacts on the nematode's metabolism and stress response

Gerrit Bredeck, Luo Zhongrui, Soledad Roig, Anna Laromaine
Institut de Ciència de Materials de Barcelona (ICMAB-CSIC), Campus de la UAB, 08193 Bellaterra, Spain,
gerrit.bredeck@hhu.de, alaromaine@icmab.es

Cellulose is one of the most frequently used additives for pharmaceutical products and in the food industry. However, impacts of this dietary fibre, that is indigestible for humans, on C. elegans have not yet been investigated. Our first goal is to explore the toxicity of cellulose such as vegetal cellulose (VC) and highly hydrated fibres of bacterial cellulose (BC). Therefore, we will assess the survival rate and growth of C. elegans upon exposure to this material1. Then we will investigate whether C. elegans takes cellulose up and if it accumulates in the intestine by optical microscopy and TEM. We will also evaluate if C. elegans

excretes cellulose or any metabolite by HPLC-MS, GC-MS and IR-spectroscopy. Lipid status is a biological parameter that could be affected by the exposition to cellulose. Literature provides data about decreased intestinal fat deposition related to life-prolonging daf-2 and daf-16 stress response signal pathway after feeding of C. elegans with grains, which are rich of insoluble fibres2,3. We strive to ascertain whether cellulose is also behaving in a similar way, since we suspect that it could trigger daf-2 and daf-16 insulin pathway. Therefore, we will evaluate the life-span of both wild-type and daf-2 knock-out respectively daf-16 knock-out strain and the lipid accumulation of those strains. With these experiments we overall expect to elucidate if cellulose acts as a dietary fibre, has an influence on the lipid metabolism and if it reduces intestinal fat deposition independent of the fact whether it is metabolized. Additionally, we will gain insight into the physico-chemical modifications that cellulose might suffer upon after exposure to C. elegans.

P-29 Epigenetic regulation of neuronal cell migration

Steffen Nørgaard, Laura Boreggio, Lisa Salcini

Biotech Research and Innovation Centre, Copenhagen University, Denmark

Throughout brain development neurons go through distinctive developmental steps. One of the critical steps for correct brain development is cell migration and numerous neurons migrate from where they are born to a target position. This process requires tight spatiotemporal regulation of various genes and mutations in genes important for neuronal migration have been connected to neurodevelopmental disorders (Liu 2011). The epigenetic status of a cell is key for correct gene expression. The chromatin factors are the ones that shapes the epigenetic landscape and thereby regulate gene activity. How chromatin factors dynamically change gene expression and thereby ensures correct cell migration in the developing brain is poorly understood. However, a growing body of evidence links mutations in several chromatin factors to brain disorders. We are currently performing a RNAi screen knocking down 270 chromatin factors in C. elegans using the Hermaphrodite Specific Neurons (HSNs) as a model for neuronal cell migration. We have confirmed that RNAi against ham-3/SMARCD3, a chromatin factor when down-regulated or mutated previously shown to perturb HSN migration (Desai, Garriga et al. 1988), causes HSN defects. We have already identified several hits important for HSN migration in our screen and are currently in the process of confirming if mutants of these genes phenocopy the RNAi phenotype. Next steps will include determining their focus-of-action, the target genes regulated and the underlying mechanisms. Desai, C., G. Garriga, S. L. McLntire and H. R. Horvitz (1988). "A genetic pathway for the development of the Caenorhabditis elegans HSN motor neurons." Nature 336: 638. Liu, J. S. (2011). "Molecular Genetics of Neuronal Migration Disorders." Current Neurology and Neuroscience Reports 11(2): 171-178.

P-31 The role of protein sumoylation in anchor cell invasion

Aleksandra Fergin, Evelyn Lattmann, Charlotte Lambert, Alex Hajnal

University of Zürich

Cell invasion plays a crucial role during normal development as well as during tumor progression, when cancer cells gain the ability to migrate through basement membranes (BMs) and spread to distant organs. To date, the mechanisms allowing cells to breach BMs remains poorly understood. During the third larval stage (L3) of the C. elegans hermaphrodite, the gonadal anchor cell (AC) breaches two BMs and invades the underlying vulval precursor cells (VPCs). Thereby, the AC establishes a connection between the uterus and the vulval epithelium. Many of the genes regulating AC invasion and the structural composition of the BM are highly conserved between nematodes and vertebrates. Therefore, AC invasion in C. elegans is a powerful in vivo model to dissect the molecular pathways controlling cell invasion. In a targeted RNAi screen to identify genes regulating cell invasion, we have found that several components of the sumoylation pathway are required for normal AC invasion, such as the E2 SUMO-conjugating enzyme ubc-9, the SUMO E3 ligase gei-17 or the SUMO ortholog smo-1. The sumoylation pathway is involved in a variety of processes including gonadal and vulval development, cell cycle progression in embryos, maintenance of chromosome structure and segregation. It is also known that many proteins are sumoylated during the epithelial to mesenchymal transition (EMT) of invasive vertebrate cells,

highlighting the importance of the sumoylation pathway in cell invasion. We have developed the tools to block protein sumoylation in a tissue-specific and temporally controlled manner in order to study how loss of sumoylation affects known regulators of cell invasion. For this purpose, we are using the tissue-specific protein degradation system to down-regulate components of sumoylation pathway (e.g. gei-17) specifically in the AC or VPCs. This approach will allow us to identify the molecular pathways through which protein sumoylation regulates cell invasion.

P-33 Clarinet (CLA-1) and the ryanodine receptor (UNC-68) interact in Ca2+ control in nerve cells.

Célia Ferreira, Marie-Anne Shaw, Ian Hope

1-School of Biology, Faculty of Biological Sciences, University of Leeds, LS2 9JT, UK. 2 - Leeds Institute of Biomedical and Clinical Sciences, St James's University Hospital, Leeds, LS9 7TF, UK.

Recognition of the role of the ryanodine receptor (RyR) in controlling neuronal endoplasmic reticulum (ER) Ca2+ storage levels and Ca2+release, and consequently neurotransmitter (NT) release, may contribute to the development of new therapeutics for neurodegenerative disorders. C. elegans has a single ryanodine receptor, UNC-68, a key intracellular Ca2+ channel for muscle and nerve cell activity. Previously, we introduced into UNC-68 single amino acid changes, equivalent to variants found in human RyR1 and responsible for myopathic conditions such as malignant hyperthermia, which confers sensitivity to anaesthetics like halothane. The modified UNC-68s confer increased sensitivity to halothane and caffeine, despite apparently fully rescuing the locomotion defect. Through a genetic screen a missense mutation and a frame-shift mutation were identified in cla-1, which further increased sensitivity to halothane specifically in the presence of the single amino acid change in UNC-68 equivalent to that in RyR1 (A4940T). This functional interaction was confirmed with independent double mutants, generated either by backcrosses or by microinjection, involving cla-1 deletion mutants. Recently CLA-1 was identified as a new active zone protein, with cla-1 mutants having reduced presynaptic NT release. Similarly, UNC-68 is necessary for normal quantal size in synaptic transmission and unc-68 mutants also display reduced NT release. We found that the interaction between cla-1 mutations and the unc-68 variant also enhances the synaptic transmission defects induced by two neuroactive drugs, aldicarb and levamisole. Aldicarb is a chemical analogue of the neurotransmitter acetylcholine, whereas the anthelmintic levamisole is a cholinergic agonist that selectively activates levamisole-sensitive acetylcholine receptors. Our results add evidence of the relevance of RyR in cellular mechanisms of neuronal function by buffering Ca2+ levels and controlling Ca2+ release during neurotransmission. Furthermore, our data challenge the assumption that human RyR variants are solely associated with myopathic conditions. Human RyR variants might also contribute to neuronal dysfunction.

P-35 Identifying new functions of the lin-3 egf/ let-23 egfr pathway through tissue-specific recombination

Silvan Spiri, Louisa Mereu, Alex Hajnal

Institut of Molecular Life Sciences University of Zurich

Epidermal growth factor (EGF)-induced activation of EGF receptor tyrosine kinases is associated with diverse processes during animal development and adulthood. Alterations of the egfr signaling network and its involvement in the progression of various types of human cancers are intensely studied. lin-3 and let-23 are the only C. elegans egf and egfr homologues. Thus, C. elegans provides a simple in vivo model to investigate egfr function without the possible redundancy of additional egfr family members. Our goal is to systematically identify tissue-specific functions of LIN-3 and LET-23 at different stages of development. The best-characterized function of LIN-3 is during vulval development, when the gonadal anchor cell (AC) releases LIN-3 to induce the primary (1°) cell fate in the adjacent vulval precursor cell (VPC) P6.p. However, after vulval induction lin-3 is also expressed in the inner-most 1° VulF cells to determine the uv1 subfate of ventral uterine and possibly also other cells. Using an endogenous let-23::gfp reporter, we observed LET_23 expression not only in the uv1 precursors but also in the AC from the mid-L3 stage on. Due to the essential roles lin-3 and let-23 play during early larval development and vulva induction, it is difficult to investigate their later functions during vulval morphogenesis. Thus, we have inserted FRT recombination sites into the lin-3 and let-23 loci to generate conditional Flp-out alleles, allowing us

to manipulate these two essential genes in a precise spatial and temporal manner. Both FRT alleles exhibit the known vulval phenotypes upon tissue-specific Flp expression. Using these tools, we are currently examining the effects of LIN-3 expressed by the vulF cells on LET-23 activation in the AC during vulval morphogenesis. Furthermore, the existing Flp driver lines permit us to systematically analyze the tissue specific functions of the lin-3/let-23 pathway in other tissues.

P-37 Involvement of the human lipodystrophy protein SEIPIN in Caenorhabditis elegans eggshell formation and early embryonic development

Sheng-Wen Chen, Leng-Jie Huang, Chao-Wen Wang

Institute of Plant and Microbial Biology, Academia Sinica, Nankang, Taipei, TAIWAN

The conserved organelle lipid droplet (LD) stores and metabolizes neutral lipids in response to diverse stimuli to maintain cellular lipid homeostasis. In the multicellular organism, such as Caenorhabditis elegans, how the LD-involved processes contribute to the growth and development of the animal remains largely unknown. Herein, we tackle this important problem by analyzing seipin, a conserved protein known in a variety of systems to control lipid droplet assembly. Our studies indicate that the C. elegans protein R01B10.6/SEIPIN forms puncta largely residing within the endoplasmic reticulum/LD contact sites, a pattern reminiscent of that in yeast and mammals, and rescues aberrant LD morphology when expressed in yeast. When seipin is deleted from the worm genome, the animals laid eggs with the level comparable to the wild-type, but only ~20% of the embryo eventually hatched. Once hatched, the subsequent development appeared normal. Neutral lipid staining and electron microscopy data revealed that abnormal LDs were accumulated in the hermaphrodite gonad, but not in the major fat storage tissues such as intestine and hypodermis, indicating that the seipin-/- animal orchestrated perturbed lipid metabolic programs in the gonad. By examining various aspects of gonad development, we conclude that the reproductive defect of the mutant is linked to altered eggshell integrity and early embryogenesis during the gastrulation and elongation stages. Hence, we propose that LD dynamics plays a pivotal role for the two developmental programs.

P-39 GCY-22 acts in salt-sensing signalling pathway at the tip of the ASER neuron cilium

Servaas van der Burght[1], Suzanne Rademakers[1], Jacque-Lynne Johnson[2], Michel Leroux[2], Gert Jansen[1]

[1]Dept. of Cell Biology Erasmus MC

[2]Dept. of Mol. Biol. and Biochem. Simon Fraser University

C. elegans senses salts in its environment using the ASE neurons. The ASEL neuron shows Ca2+ fluxes when exposed to an increase in NaCl concentration, whereas ASER responds to a decrease in NaCl concentration. The molecular mechanisms that mediate NaCl detection are not completely understood, but cGMP signalling plays an important role. In this pathway, the receptor type guanylate cyclase GCY-22 is thought to act as Na+ or Cl- receptor. We found that GCY-22, only expressed in ASER, localizes to the cilium tip and at its base in the periciliary membrane compartment (PCMC). Our goal is to understand the molecular mechanisms that regulate its trafficking and unique localization. We generated full-length eGFP and split (sp) GFP11 gcy-22 knock-ins. As expected, both GCY-22::eGFP and complemented GCY-22::spGFP localized at the tip and PCMC of the ASER cilium. Furthermore, we observed GCY-22::GFP structures in the cell body (probably Golgi) and motile vesicles in the dendrites. Using time lapse microscopy, we observed moving GCY-22::eGFP particles in the cilium, most likely transported by the intraflagellar transport (IFT) machinery. We are currently testing IFT mutants and performing dual-colour imaging to test if GCY-22::eGFP is indeed transported by IFT. FRAP experiments showed very slow recovery of GCY-22::eGFP fluorescence at the tip or PCMC, indicating the presence of a relatively stable pool of GCY-22::eGFP at these locations. However, we found quick recovery within the cilium tip or PCMC, suggesting free diffusion of the protein within these domains. To identify proteins that regulate the localization of GCY-22, we have introduced gcy-22::eGFP in the daf-25(m362) mutant background. This revealed diffuse localization of GCY-22::eGFP throughout the ASER neuron, accumulation at the PCMC, but no ciliary entry. We are using the Auxin Induced Degradation system to analyse how depletion of DAF-25 in adult animals affects GCY-22::eGFP trafficking and localization.

P-41 Pro-longevity frataxin suppression in C. elegans reduces tumor formation by dampening the RAS/MAPk signaling

Anjumara Shaik[1, 2], Sara Maciej[3], Natascia Ventura[1, 2]

[1]*Leibniz Research Institute for Environmental Medicine (IUF), Düsseldorf, Germany*

[2]*Institute of Clinical Chemistry and Laboratory Diagnostic, Medical Faculty, Heinrich Heine University of Düsseldorf, Germany*

[3]*CECAD Research Center, Cologne, Germany*

Background: Frataxin is a nuclear-encoded mitochondrial protein involved in the assembly of iron-sulphur clusters and in cellular iron homeostasis (Bencze et al., 2006). Several components of mitochondrial electron transport chain contain ISC, and hence frataxin is vital for mitochondrial functionality. Severe frataxin deficiency causes a life-threatening neurodegenerative disease in humans (i.e. Friedreich's Ataxia) and leads to arrest development in Caenorhabditis elegans. On the other hand, convincing studies from our lab reported that partial frataxin suppression via RNAi significantly extends C. elegans lifespan (Ventura et al., 2005). Further studies from our lab have shown that the opposite biological consequences in response to the different degree of mitochondrial alteration, are part of a hormetic stress response. Namely, mild mitochondrial stress results in the activation of cellular compensatory processes, such as cep-1/p53-regulated autophagy, DNA repair pathways and mitophagy which concur to provide protection against moderate mitochondrial stress, eventually increasing animal resistance to stress and healthy lifespan (Ventura et al., 2009, Torgovnick et al., 2010, Schiavi et al., 2015). Aim and Results: Aging and cancer are intrinsically coupled. It is widely established that the mechanisms which are prompted to combat aging, are in many cases the same that provide an anti-cancer effect (Serrano and Blasco, 2007). This possibility encouraged us to investigate if the anti-aging effect elicited by partial frataxin inactivation might also confer tumor suppressor activity. Using C. elegans as a model to study cancer we have found that frataxin silencing attenuates the deleterious effects, conceivably in a tissue-specific manner, and prevents premature organismal death caused by let-60/Ras-induced tumorigenic mutations (often leading to cancer in mammals). Collectively, our data suggest that making mitochondria work at the optimal level through partial frataxin depletion concurrently confers anti-aging and tumor suppressor activity.

P-43 Impact of a familial hyperglycemia-associated MDH2/mdh-2 mutation on insulin/IGF-1 signaling

Luca Pannone[1, 2], Prapaporn Jungtrakoon[3], Serena Pezzilli[4, 5], Antonella Marucci[5], Montserrat Porta-de-la-Riva[6], Julian Cerón[6], Lorella Marselli[7], Vincenzo Trischitta[4, 5], Alessandro Doria[3], Sabrina Prudente[5], Simone Martinelli[2], et al.

[1]*Genetics and Rare Diseases Research Division, Ospedale Pediatrico Bambino Gesù, IRCCS, Rome, Italy*

[2]*Department of Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome, Italy*

[3]*Joslin Diabetes Center and Harvard Medical School, Boston, MA 02215, USA*

[4]*Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy*

[5]*IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy*

[6]*Bellvitge Biomedical Research Institute - IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain*

[7]*Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy*

Whole exome sequencing of families with multigenerational diabetes not carrying MODY-gene mutations has led to the identification of two gain-of-function variants in the malate dehydrogenase 2 (MDH2) gene segregating with hyperglycemia in two unrelated families. MDH2 is localized in the mitochondria where it catalyzes the reversible oxidation of malate to oxaloacetate, and plays a pivotal role in the malate-aspartate shuttle that operates in the metabolic coordination between cytosol and mitochondria. To explore the functional impact of one of these mutations on the insulin/IGF-1 signaling (IIS) pathway, multiple strains were generated by CRISPR-Cas9 genome editing to mimic in the C. elegans genome the allele encoding the human c.154 C>T (p.Arg52Cys) substitution (p.His56Cys in the nematode). Animals were characterized in terms of longevity, resistance to oxidative stress, and fat content. Kaplan-Meier analysis revealed a mild but significant extended life span in mutant worms cultured in the presence of high D-glucose, which is known to decrease longevity by downregulating DAF-16 activity, as well as a slight resistance to oxidative stress induced by acute exposure to

paraquat. Moreover, BODIPY staining showed that accumulation of lipid droplets was significantly higher in mutant animals as compared to control worms. These changes resemble some of those reported in animals with defective IIS signaling, a condition that in humans predisposes to insulin resistance and/or inadequate insulin secretion. To explore the latter possibility, strains carrying the mutant allele were crossed with animals expressing a reporter for the insulin-like peptide DAF-28 (pdaf-28::GFP). By quantifying mean fluorescence intensity, we observed that mdh-2(His56Cys) animals show higher levels of constitutive DAF-28 expression/secretion compared to control worms, but cannot secrete it appropriately in response to glucose stimulation. In conclusion, C. elegans data support the role of MDH2 as a new diabetogene, whose mutations cause hyperglycemia in families presenting with multigenerational diabetes.

P-47 Establishment of signaling interactions with cellular resolution for every cell cycle of embryogenesis

Zhongying Zhao[1, 4], Long Chen[2], Vincy Ho[1], Ming-Kin Wong[1], Xiao-tai Huang[3], Hong Yan[2]

[1]Department of Biology, Hong Kong Baptist University, Hong Kong

[2]1Department of Electronic Engineering, City University of Hong Kong, Hong Kong

[3]3School of Computer Science and Technology, Xidian University, Xi'an, China

[4]State Key Laboratory of Environmental and Biological Analysis, Hong Kong Baptist University, Hong Kong, China

Intercellular signaling interaction plays a key role in breaking fate symmetry during animal development. Identification of the signaling interaction at cellular resolution is technically challenging, especially in a developing embryo. Here we develop a platform that allows automated inference and validation of signaling interaction for every cell cycle of C. elegans embryogenesis. This is achieved by generation of a systems-level cell contact map that consists of 1,114 highly confident intercellular contacts by modeling analysis and is validated through cell membrane labeling coupled with cell lineage analysis. We apply the map to identify cell pairs between which a Notch signaling interaction takes place. By generating expression patterns for two ligands and two receptors of Notch signaling pathway with cellular resolution using automated expression profiling technique, we are able to refine existing and identify novel Notch interactions during C. elegans embryogenesis. Targeted cell ablation followed by cell lineage analysis demonstrates the roles of signaling interactions over cell division in breaking fate symmetry. We finally develop a website that allows online access to the cell-cell contact map for mapping of other signaling interaction in the community. The platform can be adapted to establish cellular interaction from any other signaling pathways.

P-49 Towards a conditional bipartite gene expression system for C. elegans

Iskra Katic, Helge Großhans

Friedrich Miescher Institute for Biomedical Research

Bipartite and conditional systems offer the ability to control gene expression across time and in different tissues. Recent useful additions to the toolbox have been cGAL, a GAL-4-UAS system (Wang et al., 2016), and the auxin-inducible degradation system (Zhang et al., 2015). We will present our progress in establishing a straightforward combinatorial and conditional tissue-specific expression system for C. elegans.

P-51 Mitochondrial biogenesis and mitophagy are processes that are not required for lifespan extension by axenic dietary restriction in C. elegans.

Bart Braeckman, Lieselot Vandemeulebroucke

Ghent University - Biology department - Laboratory of Aging Physiology and Molecular Evolution

The largest lifespan extension amongst dietary restriction regimens is observed when worms are cultured in axenic medium . Axenically dietary restricted (ADR) worms live twice as long compared to monoxenically cultured worms. However, the mechanisms underlying the impressive lifespan extension observed in these worms are still enigmatic. Mitochondria of axenically cultured worms appear to be heavily swollen, suggesting that they may have a key function in this type of longevity. Mitochondrial biogenesis is regulated by prohibitins which are

multimeric proteins that are linked to lifespan regulation in C. elegans. Therefore, prohibitins (such as phb-2) seem attractive candidate regulators of ADR longevity. However, phb-2 mutants did not show lifespan shortening when cultured axenically, suggesting that PHB-2 is not involved in ADR lifespan extension. To assess whether mitochondrial breakdown by mitophagy may play a role, lifespan experiments were conducted using dct-1 mutants as DCT-1 is a key mediator of mitophagy. In addition, we checked whether general autophagy is involved by including hlh-30 mutant worms in our lifespan experiments. HLH-30 regulates autophagy and has been shown to play a key role in lifespan determination. Again, our lifespan data suggests that neither mitophagy nor autophagy seem to be essential in the lifespan doubling observed in axenic cultures.

P-53 Intrinsic and extrinsic factors influencing the cellular response to the chemotherapeutic agent cisplatin

Carmen Martínez Fernández[1], Jeremy Vicencio[1], Francisco Javier García Rodríguez[1], Alberto Villanueva[2], Ernest Nadal[2], Julián Cerón[1]

[1]Modelling human diseases in C. elegans Group. Bellvitge Biomedical Research Institute – IDIBELL Gran via 199, Hospitalet de Llobregat 08908, Barcelona, Spain

[2]Instituto Catalán de Oncología (ICO), Gran via 199, Hospitalet de Llobregat 08908, Barcelona, Spain

Platinum-based drugs are administered to roughly half of cancer patients treated with chemotherapy. Cisplatin is the most common of the platinum-based derivatives used in the clinic and it is very efficient inducing apoptosis in cancer cells. However, there are two major obstacles (i) toxicity in non-cancerous cells and (ii) innate or acquired cellular resistance to cisplatin. We have recently produced a couple of manuscripts using C. elegans to study the mechanisms of resistance to cisplatin. In Piulats et al. we identified which genes, located in a genomic region that is amplified in several human germ cell tumors (GCT), are implicated in the resistance to cisplatin. In García-Rodriguez et al., we have established a reliable method to study the influence of genes and other factors in the animal response to cisplatin. At the same time, we identified genes and pathways involved in inducing resistance against cisplatin. With this information, we are attempting to create super-resistant and super-sensitive strains to cisplatin for genetic and drug screening applications. Currently, we continue this research line focused on the extrinsic and intrinsic factors that modulate the impact of cisplatin on cell viability. We have observed that animal sensitivity to cisplatin can be altered through its diet. Thus, we are investigating which metabolic pathways modulate cisplatin-induced toxicity. Regarding intrinsic factors, we are creating mutants by CRISPR to study membrane transporters involved in the cellular intake and output of cisplatin. References: Orthoxenografts of testicular germ cell tumors uncover genomic changes associated to cisplatin resistance and identify PDMP as a re-sensitizing agent. Josep M. Piulat, Clinical Cancer Research (in press). Genetic and cellular sensitivity of Caenorhabditis elegans to the chemotherapeutic agent cisplatin Francisco Javier García-Rodríguez, et al BioRxiv https://doi.org/10.1101/233023. (under second revision in DMM)

P-55 Variability in the timing of a β-catenin pulse biases a stochastic cell fate decision

Jason Kroll, Jasonas Tsiaxiras, Jeroen van Zon

AMOLF

Stochastic cell fate decisions occur frequently during animal development. In many cases, individual cells randomly choose one cell fate out of a limited repertoire of fates, but with the relative frequency of the different possible fates tightly controlled. To address how signaling networks enable cell-autonomous stochastic decisions and the network properties that control the relative frequency of the resulting cell fates, we studied the stochastic differentiation of the C. elegans P3.p cell. We used time-lapse microscopy to measure the single-cell dynamics of two key regulators of the network, (BAR-1/β-catenin and LIN-39/Hox) while monitoring the cell fate decision. Strikingly, we detected pulsatile dynamics of BAR-1/β-catenin during the cell fate decision. Using experimental data and modeling approaches, we found that variability in the timing of BAR-1/β-catenin signaling activation was a significant noise source that biased the decision. Our results highlight that temporal aspects of Wnt signaling can provide sufficient variation in a signaling network to enable a tunable and cell-autonomous stochastic cell fate decision in a multicellular organism.

P-57 The Caenorhabditis elegans Integrator Complex. Transcription beyond the snRNAs.

Eva Gómez-Orte[1], Beatriz Sáenz-Narciso[1], Angelina Zheleva[1], Begoña Ezcurra[1], María de Toro[1], Hilde Nilsen[2], María P. Sacristán[3], Ralf Schnabel[4], Juan Cabello[1]

[1]CIBIR (Center for Biomedical Research of La Rioja), Piqueras 98. Logroño. 26006 La Rioja. Spain.

[2]Institute of Clinical Medicine, Department of Clinical Molecular Biology, University of Oslo and Akershus University Hospital, 1478 Lørenskog, Norway.

[3]Instituto de Biología Molecular y Celular del Cáncer (CSIC-Universidad de Salamanca), Centro de Investigación del Cáncer, Campus Miguel de Unamuno, Universidad de Salamanca, Salamanca 37007, Spain.

[4]Department of Developmental Genetics, Institute of Genetics, Technische Universität Braunschweig, 38106, Germany.

Gene expression is generally regulated by recruitment of transcription factors and RNA polymerase II (RNAP II) to specific sequences in the gene promoter region. The Integrator complex mediates processing of small nuclear RNAs (snRNAs) as well as the initiation and release of paused RNAP II at specific genes in response to growth factors. We show that in C. elegans, disruption of the Integrator complex leads to transcription of genes located downstream of the snRNA loci via a non-conventional transcription mechanism based on the lack of processing of the snRNAs. RNAP II read-through generates long chimeric RNAs containing snRNA, the intergenic region and the mature mRNA of the downstream gene located in sense. These chimeric sn-mRNAs remain as untranslated long non-coding RNAs, in the case of U1- and U2-derived sn-mRNAs, but can be translated to proteins in the case of SL-derived sn-mRNAs. The transcriptional effect caused by disruption of the Integrator complex is not restricted to genes located downstream of the snRNA loci but also affects key regulators of signaling transduction such as kinases and phosphatases. Our findings highlight that transcriptional read-through beyond the 3' end of the snRNA may stand behind the correlation between mutations in the Integrator complex and tumor transformation.

P-59 Conserved roles of the UFM1 cascade in C. elegans

Jens Daniel[1], Charlotte Sophia Kaiser[1], Adrian ter Steege[1], Dominique Bonneau[2], Eva Liebau[1]

[1]Department of Molecular Physiology, Institute for Animal Physiology, University of Münster, Schlossplatz 8, 48143 Münster, Germany

[2]Department of Biochemistry and Genetics, University Hospital, 49933 Angers Cedex 9, France

UFM1 is an ubiquitin-like posttranslational modifier that is present in most multicellular organisms. Although the UFM1 cascade was shown to be involved in several cellular processes such as apoptosis, stress resistance and proliferation it remains poorly understood. Recently, we characterized mutations in the activating enzyme of the UFM1 cascade as a cause of a severe form of early-onset encephalopathy. We have established C. elegans, which possesses the unique advantage of viable knock-out mutants of the cascade, as a model system for UFM1. Loss-of-function of the UFM1 cascade resulted a delayed development and decreased reproduction and lifespan. However, the stress resistance towards several stressors was significantly increased. Specifically, the IRE1 path of the UPR was found to be upregulated by the loss of UFMylation. Additionally, the worms displayed several neurological changes, such as failure of negative regulation of acetylcholine release, reduced sensing and impairment of the short-term memory. The results point to a conserved role of the UFM1 cascade in cellular stress response and the nervous system.

P-61 Regulation of Caenorhabditis elegans primordial germ cell abscission

Audrey Herrmann[1], Eugénie Goupil[1], Rana Amini[1], Jean-Claude Labbé[1, 2]

[1]Institute of Research in Immunology and Cancer

[2]Department of Pathology and Cell Biology, Université de Montréal, Montréal, Québec H3C 3J7, Canada.

Cytokinesis occurs after mitosis, when the two daughter cells are physically separated. In certain cell types however cytokinesis can be incomplete, generating cells that remain connected by a stable intercellular bridge, thus forming a syncytium. Stable intercellular bridges are a conserved feature during metazoan gametogenesis,

but their precise functions remain unknown. The C. elegans syncytial gonad represents a relevant model to study how differential regulation of cytokinesis impacts syncytial organization. In C. elegans, all germ cells originate from a unique embryonic blastomere, P4, which divides incompletely to form two primordial germ cells that remain stably interconnected, Z2 and Z3. We hypothesize that the block of P4 abscission during embryogenesis is required for proper syncytiogenesis of the larval and adult gonad. We are using live-imaging approaches to identify the step where P4 abscission is blocked and genetic analysis to identify molecular regulator(s) of this developmental process. Preliminary results indicate that the processing of midbody microtubules is normal in P4 and that the block of abscission occurs during one of the last steps in the process, possibly after the loading of ESCRT regulators. Our work will help to define the mechanisms that enable differential cytokinetic regulation during gonad development.

P-63 SEL-5 kinase role in anterio-posterior cell outgrowth and migration

Filip Knop, Marie Macůrková

Laboratory of Molecular Genetics of Development, Department of Cell Biology, Charles University, Prague, Czech Republic

During C.elegans development some cells follow stereotypic migration patterns or extend cellular processes in a defined direction. These events are regulated by several signalling pathways, among them the Wnt pathway. Migration of Q neuroblast descendants is one example of a Wnt-regulated migration. In search for novel regulators of this pathway we looked for enhancers of a weak retromer mutant vps-29. We uncovered a Ser/Thr kinase SEL-5 as a new regulator of the Wnt signalling pathway. sel-5 encodes a homolog of mammalian AP2-associated kinase (AAK-1) which plays a role in the regulation of clathrin-mediated endocytosis. We have found that sel-5 strongly enhances the QL migration defect seen in vps-29 mutants, suggesting a role for sel-5 in Wnt signalling. Site-of-action experiments point to a role of SEL-5 in the Wnt receiving cell. Tagged SEL-5 localizes into discrete puncta at or in the proximity of the plasma membrane. This suggests that SEL-5 could play a role in regulation of clathrin-mediated endocytosis similarly to its mammalian counterpart. We also found that vps-29 sel-5 double mutants have severely shortened posterior canals of the excretory cell. Mutants in Wnt pathway components lin-17/Fz, lin-44/Wnt or mig-14/Wls display an opposite phenotype - an overgrowth of the posterior excretory canals. We therefore see a positive regulation of the EGL-20/Wnt dependent Q cell migration and a negative regulation of the LIN-44/Wnt dependent extension of the excretory canals by SEL-5. We hypothesize that the opposite effect of SEL-5 on these two Wnt pathways could stem from the differential requirement for endocytosis in the canonical EGL-20 pathway and the non-canonical LIN-44 pathway. In summary, we identified a new regulator of Wnt signaling pathway, a Ser/Thr kinase SEL-5, that affects two distinct migrational and outgrowth events during C.elegans development. This work was supported by Czech Science Foundation grant 16-17966Y.

P-67 Mutation in a novel P. aeruginosa acyl-CoA dehydrogenase gene enhances host mitochondrial UPR activity and survival during infection

Mark Pellegrino, Siraje Mahmud

University of Texas Arlington Department of Biology 501 S. Nedderman Drive, Life Science Building (RM 337) Arlington, Texas USA 76019

Mitochondria are essential organelles that a mediate a broad set of functions supporting cell viability, most notably the production of cellular energy via oxidative phosphorylation. Cells use various means to support mitochondrial function including mitochondrial-nuclear signaling to recover the status of the organelle during times of dysfunction. The mitochondrial unfolded protein response (UPRmt) is one such signaling pathway that is activated during mitochondrial stress to reestablish homeostasis through the regulation of mitoprotective gene expression by the bZIP transcription factor ATFS-1. Recently, the UPRmt was found to be activated during exposure of various bacterial species including the opportunistic pathogen Pseudomonas aeruginosa. In this context, the UPRmt regulates not only the expression of genes to recover mitochondrial function but also the expression of innate immune genes as an anti-microbial defense mechanism to support host resistance during

infection. Here, we screened a P. aeruginosa transposon insertion mutant library in order to identify pathogen-associated pathways that could modify the activity of the UPRmt. Among the identified candidate genes, we find that loss of function in a novel acyl-CoA dehydrogenase significantly enhances the activity of the UPRmt compared to the parental strain. Interestingly, no differences in the activity of other cell stress response pathways were observed, suggesting a specific interaction with the UPRmt. Importantly, host survival is increased following loss of the novel acyl-CoA dehydrogenase in an ATFS-1-dependent manner. We will report on these and other new findings on its characterization.

P-69 Pim-related kinases selectively regulate sensory functions in C. elegans

Karunambigai Kalichamy[1], Kaisa Ikkala[1], Jonna Pörsti[1], Niina Santio[1], Sweta Hja[2], Carina Holmberg[2], Päivi Koskinen[1]

[1]Section of Genetics and Physiology, Department of Biology, University of Turku, 20500 Turku, Finland

[2]Research Programs Unit, Translational Cancer Biology program, University of Helsinki, 00290 Helsinki, Finland

Our group has been studying intracellular signalling regulated by the mammalian Pim family of serine/threonine kinases, and has identified many substrates and selective inhibitors for them. Recently we have expanded our experimentation to C. elegans, which has allowed us to initiate interesting studies on evolutionary conserved signalling pathways with physiological relevance. There are two Pim-related kinases in C. elegans, Prk-1 and Prk-2, which we have demonstrated to be true Pim orthologs with similar substrate specificity and sensitivity to our inhibitory compounds. Since we have observed Pim expression in the olfactory epithelium and other sensory organs in mice, we have now analysed the ability of Prks to regulate sensory signalling in C. elegans. With these studies, we have been able to demonstrate that Prks selectively regulate olfactory sensations to volatile attractants or repellants, but do not affect gustatory sensations.

P-71 Compression of the embryo is resolved by cell focussing

Christian Wartenberg, Christian Hennig, Ralf Schnabel

Institute of Genetics, Technical University Braunschweig, Braunschweig, Germany

Embryonic development has to be a robust process even under stress conditions. Environmental stress such as pressure is common in the wild as well as in the laboratory. Eggs are squeezed in the gonads of starved or old hermaphrodites or on microscope slides. Using polystyrene beads we squeezed embryos to a diameter of 20µm which leads to a misalignment of cells in the early embryo. During embryonic development these misalignments have to be corrected. Using 4D-microscopy, laser irradiation and subsequent bioinformatic analyses we show that a global cell sorting process we call cell focussing is responsible for the cellular movements. The overall movement of cells up to the premorphogenetic stage is not affected by the laser treatment of specific early blastomeres but the paths that cells follow in order to migrate to their individual regions are reduced. This reduction of migration depends not only on the specific blastomere that is irradiated, but also differs from embryo to embryo. These differences depend on the size of the irradiated cell, affecting the topology of the early embryo and the initial positioning of a cell reached by mitosis.

P-73 Using a forward genetic screen to identify suppressors of TDP-43 induced-toxicity

Mandy Koopman, Renée Seinstra, Ellen Nollen

University of Groningen, University Medical Center Groningen, European Research Institute for the Biology of Aging. Groningen, the Netherlands.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder typically characterized by the progressive loss of both upper and lower motor neurons. Over the years, more than 15 different ALS causing genes were identified with all of them having seemingly different biological functions. Despite these dissimilarities, the cellular hallmarks of ALS are strikingly similar when comparing patients with different affected genes. Characteristic cytoplasmic ubiquitin-positive inclusions, consisting of the RNA binding protein TDP-43, are found in 95% of the ALS cases. Intriguingly, a substantial part of the ALS-associated genes appears to influence TDP-43 metabolism in

a way that is characteristic for ALS: the depletion of TDP-43 from the nucleus and its enrichment in the cytosol. Here, we aim to unravel the genetic networks and suppressors that are able to modulate TDP-43 toxicity. For this purpose, we performed a highly saturated, forward genetic (EMS) suppressor screen in a C. elegans model for TDP-43-induced ALS, in which we used worm motility as a screening readout. The screen yielded 22 independent mutants that show higher motility. We are currently characterizing these mutants at both the behavioural (e.g. crawling, motility, paralysis) and molecular level (e.g. localization and phosphorylation of TDP43, mitochondrial function), to make phenotypic clusters. At the same time, we are identifying the mutated genes. In the next steps, we will try to elucidate via which mechanisms these genes contribute to reduced TDP-43 toxicity. Eventually, we would like to focus on those genes that have human orthologues in order to investigate whether they can also suppress TDP-43 toxicity in cell models. This fundamental insight is important when aiming for a better understanding of the ALS disease process, but also when exploring therapeutic interventions.

P-75 Chronic statin exposure increases the lifespan of C. elegans in a DAF-16 (hFOXO3a) dependent manner

Andreas Jahn, Gerhard Fritz, Sebastian Honnen

Heinrich-Heine-University Medical Faculty, Institute of Toxicology, Moorenstr. 5, 40225 Düsseldorf, Germany

Statins are broadly used as cholesterol-lowering agents in the clinic since 1989. Their competitive inhibition of the HMG-CoA reductase (HMGR-1) within the mevalonate pathway causes a depletion of mevalonate, a precursor for cholesterol synthesis and the prenylation and glycosylation of proteins. Recently, a cohort study showed that a decreased mortality rate in humans between age 78 – 90 correlates with statin treatment, but is independent of cholesterol levels. As C. elegans harbors the mevalonate pathway, but the branch leading to cholesterol synthesis is missing, it is a well-suited model organism to study cholesterol–independent effects of statins. Here, we show that chronic exposure of C. elegans to low doses of lovastatin or atorvastatin substantially decelerated the accumulation of age pigments, which is an established marker for biological ageing. Consistently both statins prolonged the lifespan of C. elegans. Together with further results (reduced fertility, shifted development, partial thermal stress resistance) these outcomes point to an involvement of the master regulator DAF-16. Neither lovastatin nor atorvastatin affect age pigments accumulation in a daf-16(mu86) mutant and lovastatin has no effect on the lifespan in this background. RT-qPCR shows an increased expression of JNK, a known activator of DAF-16, caused by mevalonate depletion (lovastatin). In line with that lovastatin failed to reduce age pigments and to increase the mean lifespan in a jnk-1(gk7) mutant. However lovastatin treatment in a daf-2(e1370) deficient background, which leads to a constitutive active DAF-16, still prolonged the lifespan of C. elegans about 10%. In summary, statin exposure leads to a longevity phenotype in C. elegans, which seems to be conferred via DAF-16 activated by JNK. Currently we investigate the molecular mechanism leading from HMG-CoA-reductase inhibition to DAF-16 activation. DAF-16 is a key molecule to understand the molecular mechanisms underlying the cholesterol-independent and protective effects of statins in humans.

P-77 pig-1 MELK is required for the non-random segregation of 'anti-apoptotic' potential during asymmetric NSM neuroblast division

Hai Wei, Barbara Conradt

Ludwig Maximilian University of Munich (LMU), Germany

Most of the 131 cells that are programmed to die during C. elegans development are generated through an asymmetric cell division. For example, the NSM neuroblast (NSMnb) divides asymmetrically by size and fate to produce a larger daughter, which survives and differentiates into a serotonergic motorneuron, the NSM, and a smaller daughter, which is programmed to die through apoptotic cell death, the NSM sister cell (NSMsc). Defects in asymmetric NSMnb division can affect the apoptotic fate of the smaller daughter and result in the production of additional NSM neurons. However, the mechanism or mechanisms through which asymmetric cell division affects the execution of apoptosis is incompletely understood. We found that the gene pig-1 (pig, par-1 (I)-like gene), which encodes the C. elegans orthologue of Maternal Embryonic Leucine-zipper kinase (MELK), acts in a conserved par-4 strd-1 mop-25.1, 2 pathway to affect the position and orientation of the NSMnb cleavage plane

as well as the kinetics, with which apoptotic cell death is executed in the NSMsc. The Snail-like transcription factor CES-1 has previously been shown to be present specifically in the larger NSM, where it represses the transcription of the pro-apoptotic BH3-only gene egl-1. Using a single-copy translational reporter of CES-1, we now demonstrate that a gradient of CES-1 protein is generated in the NSMnb just prior to its division. In addition, we found that pig-1 function is required for the establishment and/or maintenance of this gradient of 'anti-apoptotic potential'. Furthermore, we provide evidence in support of the model that in the context of the NSMnb lineage, pig-1 acts through nmy-2, which encodes a non-muscle myosin. Finally, we propose that one mechanism through which asymmetric cell division affects the execution of apoptosis is through the non-random segregation of pro- and anti-apoptotic factors.

P-79 The molecular basis of natural variation in oxidative stress response in Caenorhabditis elegans

Jana J Stastna[1], L Basten Snoek[2], Nell Nei[1], Yiru Wang[1, 2], Joost AG Riksen[2], Jan E Kammenga[2], Simon C Harvey[1]

[1]Biomolecular Research Group, School of Human and Life Sciences, Canterbury Christ Church University, CT1 1QU Canterbury, UK

[2]Laboratory of Nematology, Wageningen University, 6708 PB Wageningen, The Netherlands

Wild animals are adapted to survive in different niches and therefore represent a great source for natural variation studies. Most studies of C. elegans biology have been limited by the use of just one canonical strain N2 (Bristol) and its derived mutants. This potentially constrains the detection and functional analysis of allelic variants that could play major roles in determining control of complex traits. Studying natural genetic variation therefore allows for a better understanding of gene function as allelic interactions in divergent backgrounds pay important roles in determining complex traits. Stress and lifespan are closely related, with many mutations that alter lifespan also affecting stress-response and nutrient sensing. In some cases this means that resistance to multiple stressors is linked, with changes in the function of single genes altering resistance to multiple stresses. It is not however clear how natural selection shapes the relationships between such responses. Our latest work on a 4-parental recombinant inbred line (RIL) panels of wild strains of C. elegans found quantitative trait loci (QTL) that influenced two stress response traits (cold shock and oxidative stress). We found that one of the QTLs affecting cold stress survival we previously identified is partly explained by a polymorphism in gene elongation factor 2 (EF-2)-like protein eftu-2. The eftu-2 polymorphism also alters oxidative stress survival. This therefore identifies a molecular trade-off between the responses to these two stresses. Here, we present an extension of our analysis of natural variation in oxidative stress response in C. elegans.

P-81 Ufmylation in Caenorhabditis elegans

Charlotte Kaiser, Jens Daniel, Kevin Gilhaus, Sarah Hoedtke, Eva Liebau

Department of Molecular Physiology, Institute of Animal Physiology, University of Muenster, Schlossplatz 8, 48143 Muenster, Germany

Posttranslational modifications (PTM) increase the functional diversity of the proteome by the covalent addition of functional groups or proteins to selected amino acid residues. The covalent attachment of ubiquitin and ubiquitin-like proteins (UBL) is a common PTM. The significance of UBLs like SUMO or NEDD8 are already known, however a novel identified UBL called ubiquitin-fold modifier 1 (Ufm1) is a poorly characterized modification system. Ufm1 is involved in cell cycle control, cell differentiation and stress responses. In human pathology, it is associated with tumorigenesis and development and function of the nervous system. Similar to ubiquitination, Ufm1 is transferred to target proteins via an enzymatic cascade using the E1 enzyme Uba5, the E2 enzyme Ufc1 and the E3 enzyme Ufl1 (ufmylation). In comparison to other model organisms (like mouse, zebra fish and fly) a deletion of the Ufm-1 cascade in C. elegans is viable. Deletion mutants of Uba 5 and Ufc 1 have a reduced reproduction, life span and larval development. Interestingly, these deletion mutants can cope significantly better with stressors like juglone, heat, DTT or tunicamycin while being more sensitive towards cadmium stress. Via CRISPR/Cas9 we generated an Ufm-1 deletion mutant and have started phenotyping it. Furthermore, our project aims at the real crux of Ufm1 research by identifying and validating target proteins of the cascade. For this we have introduced various tags (e.g. His::cMyc double tag) at the 5'end of the ufm-1 gene. Furthermore,

we overexpressed extrachromosomal GFP::Ufm 1 in different Ufm-1 cascade mutants. Via pull down assays and mass spectrometry we will identify ufmylated target proteins.

P-83 Benzothiazepine CGP37157 extends lifespan in C. elegans worms

Paloma García-Casas, Jessica Arias-del-Val, Pilar Alvarez-Illera, Rosalba I Fonteriz, Mayte Montero, Javier Alvarez

Institute of Biology and Molecular Genetics (IBGM), Department of Biochemistry and Molecular Biology and Physiology, Faculty of Medicine, University of Valladolid and CSIC, Ramón y Cajal, 7, E-47005 Valladolid, SPAIN.

The benzothiazepine CGP37157 is a well-known inhibitor of the mitochondrial Na+/Ca2+ exchanger, but it lacks specificity and blocks also several other Ca2+ channels and transporters, including voltage-gated Ca2+ channels, plasma membrane Na+/Ca2+ exchanger and the Ca2+ homeostasis modulator 1 channel (CALHM1). CGP37157 has also shown neuroprotective effects in several in vitro models of excitotoxicity involving dysregulation of intracellular Ca2+ homeostasis. The mechanism of neuroprotection is unclear, but is probably related with some of its effects on Ca2+ homeostasis. We have studied here if CGP37157 could also induce changes in life expectance. We now report that CGP37157 extends C. elegans lifespan with a bell-shaped concentration–response, with high concentrations producing no effect. CGP37157 also extended the lifespan in eat-2 mutants (a model for caloric restriction), suggesting that caloric restriction is not involved in the mechanism. Instead, CGP37157 produced no effect in mitochondrial respiratory chain complex I nuo-6 mutants, suggesting that functional mitochondria are required for the effect. Our results provide a new connection between neuroprotection and anti-aging effects mediated by a modulator of Ca2+ homeostasis.

P-85 A Novel Strategy to Control Protein Misfolding Diseases and Aging: Molecular Mechanisms of Transcellular Chaperone Signalling in C. elegans

Jo Miles[1, 2], Patricija van Oosten-Hawle[1, 2], David Westhead[1, 2]

[1]*Faculty of Biological Sciences, University of Leeds, UK*

[2]*Astbury Centre for Structural Molecular Biology*

The heat shock response (HSR) promotes upregulation of cytoprotective chaperone proteins including HSP-70, in response to cellular stress conditions such as heat shock or RNAi-mediated knockdown of the essential molecular chaperone hsp-90. Using the model organism Caenorhabditis elegans we have shown that intestine-specific knockdown of hsp-90 leads to constitutively upregulated hsp-70 in cells of the body wall muscle, a process known as 'transcellular chaperone signalling' (TCS). To visualise TCS, we are utilising a C. elegans strain carrying the heat-inducible hsp-70p::mCherry reporter which is not expressed under basal conditions. Intestine-specific knockdown of hsp-90 by hairpin RNAi, however, results in activation of the HSR as indicated by strong constitutive expression of the hsp-70 reporter in the body wall muscle via TCS. This constitutive HSR activation via intestine-induced TCS results in increased lifespan and heat stress resistance. RNA-seq analysis and qPCR show that organismal expression of the three heat-inducible hsp-70 gene family members hsp-70, F44E5.4 and F44E5.5, are increased in this TCS-activated strain. GO term analysis of RNA-seq data has also identified that genes involved in the innate immune response are upregulated in this strain. To understand the molecular mechanisms underlying TCS, we have performed a forward genetic screen using the intestine-induced TCS strain which expresses the hsp-70p::mCherry reporter in muscle cells. From this screen, we isolated six mutants which show decreased expression of the hsp-70p::mCherry reporter in the body wall muscle. These mutants also display increased sensitivity to heat stress, indicating the loss of TCS between the intestine and muscle. Therefore, these mutants may harbour a mutation in a gene which could represent a potential genetic regulator of TCS.

P-87 H3K4 methylation regulators associated to intellectual disability control axon guidance

Benedetta Attianese, Steffen Nørgaard, Lisa Salcini

Biotech Research and Innovation Centre, University of Copenhagen

Next generation sequence approaches identified, among others factors, H3K4 methyltransferases and demethylases as commonly mutated in several intellectual disorders, including autism spectrum disorders, epilepsy and X-linked mental retardation (Kim et al, 2017). However how deregulation of histone modifications impacts the nerve system development and contributes to the aetiology of neurodevelopmental disorders remains elusive. By using C. elegans, we are investigating the neurodevelopmental role of the homologues of the H3K4 methyltransferases (set-2 and set-16) and the H3K4 demethylase (spr-5). Using PVQ neurons as a model for axon pathfinding, we found that mutant animals for set-2 and set-16 show defective axon guidance. Interesting, this defects seems to be temperature sensitive. These results, together with our previous studies on other H3K4 regulators, rbr-2 and jmjd-1.2 (Mariani et al, 2016, Riveiro et al, 2017), suggest that axon misguidance is a common trait of neurodevelopmental disorders. Future work includes the identification of the pathways in which these chromatin factors are involved and of target genes, by RNA sequencing approaches. The physiological roles of these chromatin factors in neuronal functions will be tested by functional assays.

P-89 Characterization of PID-1 complex and its role in 21U-RNA formation in C.elegans

Nadezda Podvalnaya[1], Ricardo J. Cordeiro Rodrigues[1], Sabrina Dietz[2], Falk Butter[2], Antonio M. J. Domingues[1], René F. Ketting[1]

[1]Biology of non-coding RNA, Institute of Molecular Biology, Ackermannweg 4, 55128 Mainz, Germany

[2]Quantitative proteomics, Institute of Molecular Biology, Ackermannweg 4, 55128 Mainz, Germany

The piRNA pathway is a widely-represented, small RNA-based mechanism that takes part in protecting genomes from transposon activity. It is mostly active in the germline and in early embryos. Despite the fact that many animal shave a piRNA pathway, many differences between these pathways exist. For instance, in many animals piRNAs are produced as a long precursor transcript that are processed afterwards to form mature multiple piRNAs. Whereas in C.elegans piRNAs are transcribed from individual loci that each produce a single piRNA species. These piRNA loci produce precursor transcripts of roughly 28 nt long that contain a 5' cap. These are then matured both at the 5' and 3' ends to produce a RNA of 21nt containing an Uracil at the 5'end. Hence, these mature piRNAs are called 21U-RNAs in C. elegans. Previously, our lab performed a genetic screening to find new factors essential for 21U-RNA biogenesis and function. Thus PID-1 was identified as an important factor for 21U-RNA production. In pid-1 mutants 21U precursors seem unaffected, but mature 21U-RNA levels are reduced. However, the exact process of 21U-RNA maturation and the role of PID-1 herein still remain unknown. Thus it was decided to study how exactly PID-1 influences on 21U-RNA biogenesis. We performed label-free quantitative mass spectrometry to identify novel PID-1 interactors. Most of the identified PID-1 complex members are expressed in the germline and the early embryo and are important for mature 21U-RNA formation. Our aim is to recapitulate the PID-1 complex in vitro to study 21U RNA processing at the molecular level. More specifically we will generate recombinant proteins as well mutant proteins to clarify the process of 21U RNA formation and the contribution of the individual proteins in 21U-RNA formation.

P-91 Wnt ligands regulate the asymmetric divisions of neuronal precursors in the C. elegans embryo

Shilpa Kaur, Sabrina Murgan, Pauline Mélénec, Guillaume Bordet, Pierre Recouvreux, Pierre-Francois Lenne, Vincent Bertrand

Institut de Biologie du Développement de Marseille, CNRS, Aix-Marseille University.

Wnt/beta-catenin signaling has been implicated in the terminal asymmetric divisions of neuronal precursors in both vertebrates and invertebrates. However, the role of Wnt ligands in this process remains poorly characterized. Here we used the terminal divisions of the embryonic neuronal precursors in C. elegans to characterize the role of Wnt ligands during this process focusing on a lineage that produces the cholinergic interneuron AIY. We observed that during interphase the neuronal precursor is elongated along the anteroposterior axis, and then divides along its major axis, generating an anterior and a posterior daughter with different fates. Using time-controlled perturbations, we show that three Wnt ligands (CWN-1, CWN-2 and MOM-2), which are transcribed at higher levels at the posterior of the embryo, regulate the orientation of the neuronal precursor and its asymmetric division. We also identified a role for a Wnt receptor (MOM-5) and a cortical

transducer APC (APR-1) in this process, while PCP proteins do not seem to be involved. MOM-5 is enriched at the posterior pole and APR-1 at the anterior pole of the neuronal precursor. Our study establishes a role for Wnt ligands in the regulation of neuronal precursor orientation and terminal asymmetric divisions, and characterizes the role of downstream components.

P-93 Population density affects recovery from L1 arrest via insulin signalling

Alejandro Mata-Cabana[1], Mª Jesús Rodríguez-Palero[2], Laura Gómez-Delgado[1], Marta Artal-Sanz[2], María Olmedo[1]

[1]Department of Genetics, University of Sevilla

[2]CABD, University Pablo de Olavide

All organisms face feast and famine during their life. When C. elegans embryos hatch in absence of food the animals arrest postembryonic development at the first larval stage (L1). Arrested L1s can survive several weeks without food and show increased resistance to stress. When fed, larvae resume development, but animals subjected to long periods of starvation take longer to reach adulthood. Measuring developmental timing we have noticed that this extension is due to time that animals need to recover from starvation before resuming development. High density population during L1 arrest increases survival during starvation. This density effect is mediated by secreted compounds of unknown nature. Here we show that high population density during prolonged starvation decreases recovery time. The density-dependent survival is reduced in daf-16 mutants and high density during arrest prompts a reduction in insulin production and a consequent sustained nuclear localization of DAF-16. These findings reveal that the effect of density is mediated by the Insulin/insulin-like growth factor signalling (IIS) pathway. Moreover, we have tested the disaccharide trehalose as a candidate compound responsible of the density effect on arrested L1. During starvation there is a metabolic shift towards the production of trehalose in a DAF-16-dependent manner, which supports survival. We have detected small amounts of trehalose in the supernatant of highly populated arrested L1s. The external addition of this amount of trehalose restores nuclear localization of DAF-16 in arrested L1s at high density where the endogenous trehalose was previously removed. When it is added to L1s at low-density, trehalose resembles the DAF-16 localization of animals at high density. We can conclude that high density of animals mimics low insulin signalling by increasing the nuclear localization of DAF-16, contributing to longer survival and fast recovery from arrest, and that this effect is mediated by trehalose as a possible signal.

P-95 The role of Crumbs in C. elegans larval epithelia

Victoria G Castiglioni, Mike Boxem

Developmental Biology, Department of Biology, Faculty of Science, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

Crumbs complex proteins play a central role in establishing apical domain identity in epithelial cells in Drosophila and mammals. However, recent studies in the Drosophila eye found that Crumbs has important roles in tissue morphogenesis and growth control as well. Such additional function of Crumbs are difficult to study due to the prominent role in apical-basal polarity establishment in most tissues in which it is expressed. The C. elegans genome encodes three Crumbs family members (CRB-1, EAT-20, CRB-3), like mammals. All three Crumbs proteins are expressed in multiple epithelial tissues and localize to the apical domain. However, the C. elegans Crumbs is not essential for in apical-basal polarity, and a triple deletion mutant is viable without any obvious defects. Here, we aim to gain new insights into the roles of Crumbs proteins, making use of the non-essential nature of the Crumbs family in C. elegans. We performed a forward EMS mutagenesis screen to identify genes that work with Crumbs to control epithelial polarity or morphogenesis. We identified several mutants that show synthetic lethality with a triple crb-1 eat-20 crb-3 deletion strain, and are currently identifying the causative mutations through mapping by whole-genome sequencing. Our ultimate goal is to improve our understanding of the role of Crumbs in controlling apical identity, as well as its interaction with other cellular processes important for epithelial functioning.

P-97 Functional characterization of ikb-1 and nfki-1, homologs of a negative regulator of NF-κB pathway

David Brena[1], Montserrat Porta de la Riva[1, 4], Eric Cornes[1, 5], Joan Bertran[2, 3], Lluís Campos[1], Dmytro Kukhtar[1], Albert García López[1], Anna Bigas[3], Lluís Espinosa[3], Julián Cerón[1]

[1]Modelling Human Disease in Caenorhabditis elegans, Department of Molecular Genetics, Bellvitge Biomedical Research Institute (IDIBELL), Hospital Duran i Reynals, Hospitalet del Llobregat (Barcelona), 08908, Spain

[2]Universitat de Vic, Universitat Central de Catalunya (UVic-UCC), Vic, 08500, Spain

[3]Program in Cancer Research, Hospital del Mar Medical Research Institute (IMIM), Barcelona 08003, Spain

[4]Group of Neurophotonics and Mechanical systems Biology, The Institute of Photonic Sciences (ICFO), Castelldefels (Barcelona), 08860, Spain

[5]Department of Developmental and Stem Cell Biology, Institut Pasteur, Paris Cedex 15, 75724, France

The Nuclear Factor κB (NF-κB) signaling pathway not only promotes tumorigenesis in different ways but is also involved in the mechanisms of resistance to cancer treatments. In other words, NF-κB pathway activity is usually beneficial for cancer cells and thus deleterious for patients. Though there are no NF-κB homologs in C. elegans, our favorite nematode has two homologs for IκB-α, which has been traditionally described as negative regulator of the NF-κB pathway. Such repressive activity would require the sequestering NF-κB in the cytoplasm. A publication from our collaborators (Mulero et al, 2013) uncovered in mammals an unexpected nuclear role for IκB-α that is against the dogmatic view of IκB-α functioning exclusively in the cytoplasm. Interestingly, they found that nuclear IκB-α regulates a subset of polycomb genes in differentiation and cancer. We are investigating the activity of nfki-1 and ikb-1, which are the two C. elegans homologs of mammalian IκB-α, to better understand its nuclear functions. First, we generated reporter strains (NFKI-1::GFP and IKB-1::mCHERRY) and found that both NFKI-1 and IKB-1 are present in the nucleus of several cell types. Although deletion mutants for nfki-1 and ikb-1 do not show any obvious phenotype (just some morphological defects at very low penetrance), RNA-seq analyses uncovered upregulation of germline genes, suggesting a role of these two proteins in repressing germ cell fate. We found that NFKI-1 and IKB-1 are associated to chromatin, and ChIP-seq experiments showed that nfki-1 and ikb-1 mutants modify the landscape of the chromatin histone marks H3K27me3 and H3K36me3. We are currently studying the role of nfki-1 and ikb-1 in the maintenance of the cell fate in distinct cell types. Reference: Mulero et al, Cancer Cell. 2013 Aug 12;24(2):151-66.

P-99 Using lineage-tracing to study how histone methylation regulates embryonic cell fate decisions

Juan D. Rodriguez, Brandon Carpenter, David J. Katz

Department of Cell Biology Emory University

Genetic and epigenetic information are transmitted from one generation to the next through the germline. Although the heritability of genetic information is stable from one generation to the next, epigenetic information is highly modified within each generation to regulate proper gene expression. After fertilization, each embryo must reprogram their epigenome and reestablish an epigenetic ground state to allow normal development to proceed. In C. elegans, two epigenetic enzymes, the H3K4me2 demethylase, SPR5, and the H3K9 methyltransferase, MET-2, are maternally deposited into the oocyte and cooperate to reestablish the epigenetic ground state by modifying histone methylation. Progeny of worms lacking spr-5 and met-2 accumulate high levels of H3K4me2 within two generations, resulting in complete sterility and improper somatic expression of spermatogenesis genes. However, the precise developmental consequences of misregulating germline/soma identity in embryos are unclear. To interrogate how reprogramming defects may affect early embryonic development, we are using confocal imaging to perform automated lineage tracing experiments. The C. elegans embryonic lineage is normally invariant. I will investigate how inappropriate inheritance of histone methylation affects cell fate specification in the early embryo. By identifying defects in the embryonic lineage, we hope to gain mechanistic insight into the consequences of improper germline/soma identity. We hypothesize that an abnormal accumulation of histone methylation could affect cell fate, cause abnormal cell divisions, and perhaps lead to inappropriate cell death.

P-103 Dissecting centriole elimination during embryogenesis

Nils Kalbfuß, Marie Pierron, Pierre Gönczy

École Polytechnique Fédérale de Lausanne

Centrosomes, consisting of centrioles and pericentriolar material, are the principal microtubule-organizing centers of animal cells. In proliferating tissues, centrosomes are critical for countless fundamental cellular processes during interphase and direct spindle assembly during mitosis. Upon exit from the cell cycle and differentiation, centrioles can become basal bodies crucial for cilia formation in some cells. In other differentiating tissues, centrioles are thought to be eliminated. However, the extent to which this is the case and the potential underlying mechanisms are not known. I aim at creating a "centriole elimination map" in C. elegans embryos to uncover the fate of centrioles throughout C. elegans embryogenesis. Imaging live embryos using light sheet microscopy, as well as fixed specimen using highly resolving confocal microscopy, we found that centrioles, as marked by GFP::SAS-7, start to disappear in some cells after the bean stage, overlapping with the transition from the proliferation to the morphogenesis phase of embryogenesis. This work sets the foundation for a comprehensive assessment of centriole fate during C. elegans embryogenesis, and an investigation of the underlying mechanisms.

P-105 Modelling human cancer SF3B1 mutations in C. elegans

Xènia Serrat[1], Anna Esteve[2], Sol Katzman[3], Alan Zahler[3], Julián Cerón[1]

[1]*Modelling human diseases in C. elegans Group. Bellvitge Biomedical Research Institute – IDIBELL Gran Via 199, Hospitalet de Llobregat 08908, Barcelona, Spain.*

[2]*CNAG-CRG, Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), Baldiri i Reixac 4, 08028, Barcelona, Spain.*

[3]*Department of Molecular, Cell, and Developmental Biology and The Center for Molecular Biology of RNA, University of California Santa Cruz, Santa Cruz, California 95064, USA*

SF3B1 is the most commonly mutated splicing factor in different cancer types, and in particular in hematological malignancies. Mutations in this gene are missense mutations and cluster in conserved residues in HEAT domains. Although different amino acid changes are associated to particular cancer types, their common overall effect is a SF3B1 change-of-function resulting in aberrant 3'SS selection. Taking advantage of the high level of conservation of the mutated residues, we introduced in C. elegans by CRISPR/Cas9 the K700E mutation, which is the most prevalent. Whereas a sftb-1/SF3B1 null mutation produces a developmental arrest, sftb-1(cer7[K718E]) animals do not show any obvious phenotype and present very slight defects in alternative splicing. Strikingly, we discovered an additive effect by combining this and two other prevalent point mutations. Indeed, sftb-1(cer7[K718E]; cer16[R643C]; cer17[Q552P]) triple mutant worms display developmental delay and fertility problems. We subjected the three single mutants to an RNAi screen searching for genetic interactions with other splicing factors and identified U2 snRNP components as potential genetic interactors. All together, we present C. elegans as a system to study the pathogenic mechanisms of SF3B1 mutations and to investigate pharmacological vulnerabilities of these cancer mutations.

P-107 Mapping a novel oxidative stress response pathway involving NHR-49

Kelsie Doering, Zoe DeBoer, Grace Goh, Stefan Taubert

Medical Genetics, University of British Columbia; Centre for Molecular Medicine and Therapeutics, BC Children's Hospital

Organisms encounter many harmful environmental stresses. Thus, an organism's ability to mount specific stress responses is critical for survival. Oxidative stress occurs when reactive oxygen species (ROS), obligate and ubiquitous by-products of aerobic respiration, accumulate within the cell to toxic levels. The pathways that regulate the cell's response to oxidative stress are evolutionarily conserved from Caenorhabditis elegans to humans, and in C. elegans typically require SKiNhead-1 (SKN-1). SKN-1 is considered a master regulator of oxidative stress responses which is critical in the control of cellular responses to, and the defense against this stress. Though the responses controlled by this master regulator is considered the principal pathway, evidence

for parallel programs exist. Indeed, our recent data shows that the transcription factor Nuclear Hormone Receptor NHR-49, which functions in lipid homeostasis, is required for (a) worm survival to the oxidative stressor tert-butyl hydroperoxide (tBOOH); and (b) to induce skn-1-independent transcriptional responses to this stress, including induction of the tBOOH response gene fmo-2. To identify new players acting in this pathway, I am performing a reverse genetic screen using RNAi. As readout, I am using a transcriptional reporter, composed of the GFP reporter fused to the promoter of fmo-2. Targeted RNAi will identify which of the C. elegans transcription factors, kinases, and transcriptional co-regulators are required for tBOOH-dependent induction of fmo-2, and thus might map into the NHR-49 pathway.

P-109 Effects of autophagy gene downregulation on the ubiquitin-proteasome system in C. elegans.

Sweta Jha, Carina Holmberg-Still

Research Programs Unit, Translational Cancer Biology, University of Helsinki, Finland

The ubiquitin-proteasome system (UPS) and the autophagy-lysosomal pathway (ALP), the two main proteolytic mechanisms in eukaryotic cells, play crucial parts in maintaining protein homeostasis as well as in the maintenance of amino acid pools and energy balance. Dysfunctions of these system have been implicated in the pathogenesis of many age-related diseases such as neurodegenerative and autoimmune diseases, as well as different types of cancer. Recent studies indicate that there is an interplay between them. Here, we study the tissue-specific effect of autophagy genes on UPS at the organismal level using C. elegans. We use a combination of in vitro approaches, genetics and transgenic C. elegans expressing various fluorescent reporter proteins. For example, for UPS analyses we have previously developed a photoconvertible UPS reporter system, which measures protein degradation independently of translation of new proteins, and a fluorescent polyubiquitin reporter reflecting the endogenous pool of Lys48-linked polyubiquitinated proteasomal substrates(1). Our data show that downregulation of some autophagy genes decreases proteasome activity in tissue-specific manner both in vivo and in vitro without affecting the total abundance of proteasome. On the other hand, RNAi of other autophagy genes decreases the total amount of proteasome and also decreases proteasome activity but only in intestinal cells. Therefore, my preliminary data suggest that autophagy genes affect proteasome in a tissue-specific manner. A better understanding of the link(s) between UPS and ALP will provide new and important understanding on tissue-specific regulation of protein homeostasis in an animal. Reference: 1. Matilainen O., Jha S., and Holmberg C.I. (2016). Fluorescent tools for in vivo studies on the ubiquitin-proteasome system. Methods Mol Biol. 1449:215-222.

P-111 The role of the RhoGEF ect-2 in RAS/MAPK-induced germ cell death

Tea Kohlbrenner[1], Simon Berger[1, 2], Kirsti Arumäe[1], Tinri Aegerter-Wilmsen[1], Xavier Casadevall i Solvas[2], Andrew deMello[2], Alex Hajnal[1]

[1]*Institute of Molecular Life Science, University of Zürich*

[2]*Institute for Chemical and Bioengineering, Department for Chemistry and Applied Biosciences, ETH Zürich*

Cell death in the Caenorhabditis elegans hermaphrodite germline is a physiological process ensuring cell homeostasis during oogenesis. Around half of the germ cells die instead of maturing into oocytes. In contrast to the programmed cell death in the soma (apoptosis), germ cell death appears to occur randomly. Previous studies have shown that activation of the Ras/MAPK pathway in pachytene stage germ cells is not only necessary for pachytene exit but also for germ cell death. Mutations that increase RAS/MAPK signaling lead to the production of smaller oocytes and an elevated rate of cell death, whereas mutations that inactivate the RAS/MAPK pathway block germ cell death and oogenesis. By performing live imaging of germ cells, we observed that germ cells size decreases before cell death. Hyper-activation of the RAS/MAPK pathway results in an elevated number of small germ cells. This accumulation of smaller cells is independent of the CED cell death pathway, indicating that decreased cell size is not only caused by activation of the CED pathway. Germ cell size is largely controlled through constriction of the apical membrane domain and driven by the actomyosin network. Blocking apical germ cell constriction by inhibiting the non-muscle myosin NMY-2 or its upstream activator, the RhoGEF ECT-2, reduces the rate of germ cell death in the wild-type as well as in the let-60 ras(ga89) gain-of-function background.

Conversely, the ect-2(zh8) gain-of-function mutation (Canevascini et al. 2005) reduces germ cell size, while simultaneously increasing the rate of cell death. Preliminary experiments indicate that RAS/MAPK signaling reduces germ cell size by activating the RhoGEF ECT-2 in pachytene stage germ cells. We therefore propose that RAS/MAPK signaling regulates germ cell size through the actomyosin network and that cell size is one determinant of germ cell death.

P-113 Study of development into a diapause stage in C. elegans

Daisy S. Lim, Nari Kim, Junho Lee
School of Biological Sciences, Seoul National University, Seoul 08826, Republic of Korea

Under adverse environmental conditions, such as high population, high temperature and scarce food, C. elegans is able to develop into an alternative diapause development stage called dauer. Specialized for survival in harsh conditions, dauer stage is commonly found in the wild and conserved in diverse nematode species, implying its importance in survival of the species. Many studies from last decades have revealed environmental factors and related signaling pathways that affect dauer entry. However, the downstream factors that directly affect dauer development are yet to be identified. We have discovered a recessive C. elegans mutant that is otherwise fertile but lethal in various dauer-inducing conditions. This mutant develops normally until the dauer entry, but becomes trapped in its dauer molt and eventually dies. Using SNP Mapping-by-Sequencing approach with Hawaiian wild isolate CB4856, we were able to map the causative mutation to the central region of chromosome IV. Identification of the causal mutation and its genetic mechanism that leads to this stage-specific phenotype will further our understanding of dauer development and regulation of developmental plasticity in C. elegans.

P-115 The role of basolateral polarity regulators in epithelial tissue homeostasis

Amalia Riga, Helena Pires, Victoria Garcia, Mike Boxem
Utrecht University, Developmental Biology, Padualaan 8, 3584 CH Utrecht

Polarization of epithelial cells into apical and basolateral domains is essential for the functioning of epithelia as selectively permeable barriers. Loss of epithelial polarity contributes to diseases like polycystic kidney disease and retinal dystrophies. Moreover, epithelial cancers are characterized by loss of cell polarity and epithelial integrity. The basolateral Scribble proteins (Scrib, Lgl, Dlg) were originally identified as tumor suppressors in D. melanogaster and later found to regulate epithelial polarization and junction formation. However, the actual role of these proteins in established epithelia and their functional relationships are still far from understood, and likely vary between cell types. Moreover, how Scribble proteins promote malignant transformation of cells is not yet elucidated. Here, we use C. elegans to study with single cell resolution the requirements of Scribble proteins in epithelial tissue homeostasis and their interactions with different polarity regulators. In C. elegans, loss of LET-413/Scrib and DLG-1/Dlg results in defects in epithelial polarization and junction formation during embryonic development and causes embryonic lethality. To study the role of these proteins in established larval epithelia, we generated alleles that allow inducible protein degradation. In contrast to earlier results obtained by RNAi, we find that elimination of LET-413 results in a developmental arrest. We are currently following the effects of LET-413 inactivation on the polarity machinery, junctions and tissue integrity, using live-cell microscopy.

P-117 The role of ribosomal DNA copy number in C. elegans ageing

Andre Zylstra, Olivia Casanueva, Jonathan Houseley
The Babraham Institute, United Kingdom

Ribosomal DNA (rDNA) instability shortens lifespan in yeast replicative aging models and it has recently been shown that the rate of copy number change is attenuated under caloric restriction or mTOR inhibition. Furthermore, heritable diet-dependent copy number changes have been reported in Drosophila, dependent on mTOR signalling, while rDNA copy number correlates with transcriptomic variation in both flies and human cell lines – suggesting there may be functional consequences at the level of gene expression. These results led us to ask whether rDNA copy number variation influences metazoan aging, using the nematode Caenorhabditis

elegans as a model. We have developed two methods for determining rDNA copy number in C. elegans based on qPCR and pulsed field gel electrophoresis which produce highly concordant copy number estimates. Testing several long-lived strains revealed that all had much larger rDNA arrays than wild type N2 populations. To further analyse the separate effects of these mutations and rDNA copy number variation we performed a genetic cross and are analysing the progeny populations for rDNA copy number plasticity, fitness and lifespan.

P-119 DAF-21/Hsp90 is required for C. elegans longevity by ensuring DAF-16/FOXO isoform A function

Milán Somogyvári, Eszter Gecse, Csaba Sőti

Semmelweis University, Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Budapest, Hungary

The FOXO transcription factor family is a conserved regulator of longevity and the downstream target of insulin/insulin-like signaling (ILS). In Caenorhabditis elegans, the FOXO ortholog DAF-16A and D/F isoforms extend lifespan in daf-2 insulin-like receptor mutants. Here we identify DAF-21/Hsp90, a central facilitator of proteostasis and signaling, as a novel regulator of longevity. We find that reducing DAF-21 capacity by daf-21(RNAi) inititated either at the beginning or at the end of larval development, respectively, shortens wild-type lifespan. Further, daf-21 knockdown employed from the beginning, but not from the end, of larval development, decreases the longevity of daf-2 mutant and daf-2 silenced nematodes. daf-16 loss of function mitigates the lifespan shortening effect of daf-21 silencing. Consistent with this, we demonstrate that DAF-21 promotes daf-2 and heat shock induced nuclear translocation of DAF-16A as well as the induction of DAF-16A-specific mRNAs. In contrast, daf-21(RNAi) neither influences the localization of DAF-16D/F, nor the expression of its target genes. DAF-21 is dispensable for the protein stability and nuclear import of DAF-16A, excluding a chaperone client interaction and suggesting that DAF-21 regulates DAF-16A activation upstream of its cellular traffic. Finally, we show a selective requirement for DAF-21 to extend lifespan of DAF-16A, but not DAF-16D/F, transgenic daf-2 mutant strains. Our findings indicate that DAF-21 functions from larval development to ensure wild-type and dampened ILS induced longevity and reveal an isoform-specific regulation of DAF-16 activity.

P-121 Benzaldehyde-induced aversion and adaptive cellular responses in C. elegans

Gábor Hajdú[1], István Taisz[1, 2], Csaba Sőti[1]

[1]*Semmelweis University, Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Budapest, Hungary*

[2]*Current affiliation: MRC Laboratory of Molecular Biology, Neurobiology Department, Cambridge, UK*

In response to tissue damaging noxae, conserved cellular stress responses, xenobiotic detoxification and aversive behaviour facilitate organismal survival. The nematode Caenorhabditis elegans exhibits a concentration dependent biphasic behavioural response to the food ingredient volatile benzaldehyde (BA). Concentrated BA evokes an initial attraction followed by a strong aversion. However, whether aversion is a consequence of chemosensation or direct tissue damage is unknown. In this study we investigate BA-dependent toxicity, the protective cellular responses and their relationship to the aversive behaviour. We demonstrate that BA-treatment induces dose dependent paralysis, death, and reduces thermotolerance. At the cellular level, exposure to BA specifically triggers nuclear translocation of the stress-responsive DAF-16/FOXO transcription factor and elevated expression of the DAF-16 target phase-I enzyme cyp-35b::GFP and the SKN-1/Nrf2 target phase-II enzyme gst-4::GFP detoxification reporters. Further, BA robustly increases autofluorescence of the intestinal aging marker Lysosome Related Organelles (LRO). Interestingly, experiments using LRO biogenesis and detoxification deficient mutants reveal that LRO-granules are required for survival and thermotolerance upon BA exposure. Currently, employing RNAi tools and mutants of various surveillance mechanisms, including DAF-16/FOXO, DAF-21/Hsp90 and SKN-1/Nrf2 induced stress and detoxification processes, we test how the abovementioned protective cellular responses affect BA-induced avoidance and adaptation. Our work beyond providing the first evidence on the protective role of C. elegans LRO-s may unveil a link between adaptive cellular responses and benzaldehyde induced aversion.

P-123 Characterization of the Capicua homolog GEI-3 in C. elegans

Laura Rodríguez-Muñoz[1], Xènia Serrat[2], Julián Cerón[2], Gerardo Jiménez[1, 3]

[1]*Gene expression and signaling Group. Molecular Biology Institute of Barcelona (IBMB)-CSIC, 08028, Barcelona, Spain*

[2]*Modelling human diseases in C. elegans Group. Bellvitge Biomedical Research Institute – IDIBELL, 08908, Hospitalet de Llobregat, Barcelona, Spain*

[3]*Institució Catalana de Recerca i Estudis Avançats, 08010, Barcelona, Spain*

The HMG-box protein Capicua (Cic) is a conserved transcriptional repressor that functions downstream of the receptor tyrosine kinase (RTK)-Ras-MAPK signaling pathway. Cic and MAPK signaling function antagonistically in a relatively simple molecular switch: in the absence of signaling, Cic represses genes regulated by MAPK signaling, whereas upon MAPK activation, Cic is phosphorylated and downregulated and this leads to derepression of its target genes. Initially described in Drosophila development, this switch is also conserved in mammals, where Cic has been implicated in neurodegeneration and functions as a tumor suppressor. The precise roles of CIC in development and disease remain, however, incompletely understood and we reasoned that C. elegans should provide a complementary model for their study. Similarly to Drosophila and mammals, C. elegans has a single cic ortholog, gei-3, which remains genetically uncharacterized. Furthermore, all these species express both short (Cic-S) and long (Cic-L) isoforms of the protein, the functional significance of which remains unclear. To begin the characterization of gei-3, we have used CRISPR-Cas9 to completely eliminate the sequences encoding both Gei-3-S and -L isoforms. We find that homozygous worms carrying this deletion are egg-laying defective and show a protruding-vulva phenotype in 40% of the animals at 20ºC. We are currently dissecting the origin of these defects and we are also generating two additional mutations: (i) a gei-3-L-specific allele, and (ii) an in-frame deletion that removes a conserved docking site for MAPK, a mutation that produces MAPK-insensitive, constitutively active forms of Cic in Drosophila. These various alleles should allow us to explore the potential role(s) of GEI-3 downstream of MAPK signaling.

P-125 Three pathways regulate spindle directions in three dimensions

Tania Sastradihardja[1], Christian Hennig[1], Frank Eggert[2], Ralf Schnabel[1]

[1]*Institute of Genetics, Technical University Braunschweig, Braunschweig, Germany*

[2]*Institute of Psychology, Technical University Braunschweig, Braunschweig, Germany*

Proper cleavage directions of blastomeres are essential for early embryonic development of C. elegans. Therefore, cleavage directions are highly regulated. The Wnt pathway and LAT-1, a G protein coupled receptor, were already identified as regulators of the cleavages of the four AB derived blastomeres at the eight cell stage embryo. However, we expect that more than two pathways are required to define the orientation of a spindle in three dimensions. Indeed we identified a third pathway, the Fibroblast Growth Factor (FGF) pathway, playing a role in the regulation of spindle alignment. FGF is already known to regulate sex myoblast migration and several other important processes. To investigate the individual contribution of all three pathways on spindle direction we used a, for this type of problem, new bioinformatics approach. The cell division coordinates of wildtype and mutant embryos are plotted in a 3D coordinate system and then their distributions are investigated by a variation of the Principle Component Analysis. We speculate that the differences of the main variance axes between wildtype and mutant embryos indicate the direction specified by a respective pathway. Often only hypomorphic but not amorphic activity reductions are available, thus using the main variance axes may facilitate to overcome this common problem. The differential spindles directions of the four AB derived blastomeres upon manipulation of the three polarity pathways indicate that all spindles are regulated individually i.e. the four spindles behave differently in respect to each other in all three polarity pathways.

P-127 Investigating the conserved mechanosensory function of C. elegans tmc-1

Eva Kaulich, William R Schafer

Division of Neurobiology, MRC Laboratory of Molecular Biology, Cambridge Biomedical Campus, Francis Crick Avenue, Cambridge CB2 0QH, UK

The conserved family of Transmembrane channel-like (TMC) proteins has attracted a lot of interest since two members appear to be key components of the mammalian hair cell mechanotransducer involved in hearing. However, it is still unclear what role TMCs play in these complexes, whether they are channel proteins, and how they participate in the detection of sensory stimuli. C. elegans expresses two TMC proteins, tmc-1 and tmc-2. While tmc-2 seems to be exclusively expressed in the muscles, tmc-1 is also widely expressed in the nervous system. This wide expression pattern suggests that tmc-1 might serve different functions in the various neurons. Until recently, tmc-1 function C. elegans neurons was only described to play a role in chemosensation. However, we and others have identified tmc-1 expression in C. elegans neurons involved in mechanotransduction, such as the high-threshold mechanotransducer ALA. The expression of tmc-1 in ALA gives us the chance to study the conserved function of TMCs in mechanosensation in vivo. By using calcium imaging in a microfluidic chip and behavioural egg-laying assays, we are establishing the function of tmc-1 within ALA. Finding a robust phenotype will then allow us to investigate the tmc-1 containing sensory transduction complex composition using genetic and molecular approaches to identify genes whose products functionally interact with tmc-1. This project's aim is to understand the function and composition of the tmc-1 in the transduction complex, ultimately transferring the acquired knowledge from C. elegans to higher systems.

P-131 Penetrance of the Weismann barrier: An endoribonuclease in the soma protects germline immortality in C. elegans

Ralf Baumeister[1], Wolfgang Maier[1], Dietmar Pfeifer[2], Erika D von Gromoff[1]

[1]Bioinformatics and Molecular Genetics (Faculty of Biology), Center for Biochemistry and Molecular Cell Research (Faculty of Medicine), University of Freiburg, Germany

[2]Core Facility Genomics, Department of Internal Medicine I, and Division of Pediatric Infectious Diseases and Rheumatology, Centre of Paediatrics and Adolescent Medicine, University Medical Center Freiburg

125 years ago, August Weismann introduced the Weismann barrier theory between the germline and somatic cells. According to this theory, environmental information can only flow from the germ cells to the soma but not vice versa. Even though recent studies in epigenetics have indicated that parental exposure to environmental stress can modify progeny physiology, the mechanisms regulating these phenomena are poorly understood. In a genome-wide screen looking for genes facilitating germline tumor formation, we identified endu-2 as important participant of the tumorigenesis in C. elegans. endu-2 encodes a conserved poly-U specific endoribonuclease and loss of endu-2 causes a temperature dependent gradually loss of germline immortality over generations. Expression pattern analysis shows that ENDU-2 is expressed in the somatic tissues. However, ENDU-2 is secreted from the soma and uptaken by the germline in a temperature dependent manner and this is essential for maintaining germline immortality of animals. Sequencing of co-immunoprecipitated RNA of ENDU-2 reveals that ENDU-2 binds to mRNA, including mRNA of multiple histone modifiers. In summary, our data suggest that ENDU-2 may penetrate the Weismann barrier to mediate epigenetic information flowing from the soma to the germline. We are currently addressing role of ENDU-2 in controlling gene expression. The latest results will be presented in the meeting.

P-133 How does the non-canonical Wnt receptor CAM-1/Ror2 control neuroblast polarity?

Christa van der Veen, Lorenzo Rella, Hendrik Korswagen

Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences (KNAW) and University Medical Center Utrecht (UMCU)

The Wnt family of secreted signaling proteins plays an important role in development and disease. It controls cell fate decisions and stem cell maintenance through beta-catenin dependent regulation of gene expression (the canonical pathway), but also signal independently of beta-catenin (non-canonical) to regulate cell and axon migration. Non-canonical Wnt signaling can be triggered through binding of Wnt ligands to members of Frizzled (Fz) Wnt receptor family, but also by receptors like the receptor tyrosine kinase Ror2. Deregulation of non-canonical Wnt signaling plays an important role in cancer cell invasion and metastasis, yet how non-canonical Wnt pathways control migration is poorly understood. The highly stereotypic migration of the C. elegans QR

neuroblast descendants provides a powerful model to study Wnt dependent cell migration in vivo. We have recently shown that migration of the QR descendant QR.p is dependent on two parallel acting, non-canonical Wnt pathways: a MOM-5/Fz dependent pathway controlling the speed of migration and a CAM-1/Ror2 dependent pathway that is important for the correct polarity of the cells. We are interested in how the CAM-1/Ror2 pathway controls cell polarity. CAM-1/Ror2 may control polarity by directly interphasing with the cellular polarity machinery, but there is also evidence that the Ror2 pathway may control polarity through transcriptional regulation of specific target genes. To distinguish between these possibilities, we are isolating QR neuroblast descendants from control and cam-1 mutant animals and are examining gene expression differences through RNA sequencing. Furthermore, we are developing a system for Q neuroblast-specific RNAi that will enable screening for new downstream pathway components of the CAM-1/Ror2 pathway.

P-135 Role and regulation of the MT-severing enzyme Katanin in C. elegans

Nicolas Joly, Eva Beaumale, Lucie Van Hove, Lionel Pintard

Cell Cycle and development Team, CNRS-UMR7592, Institut Jacques Monod, Paris, France.

Microtubules (MTs) are dynamic cytoskeletal polymers with instrumental functions in cell division (meiosis and mitosis), morphogenesis, motility and signaling. MTs constantly polymerize and shrink and this dynamic behavior, which is critical for their function, is regulated by a large family of MT-associated proteins (MAPs). Whereas most of these MAPs interact with the microtubule plus or minus ends, another class interacts with the MT lattice and severs MTs along their length, thereby controlling MTs size and density. Three evolutionarily conserved AAA+ (ATPase Associated with diverse cellular Activities) MT-severing enzymes have been identified: Fidgetin, Spastin and Katanin. Mutation of these enzymes has been linked to various defects and pathologies including developmental defects, neurodegenerative disorders such as hereditary spastic paraplegia (HSP), Fidget disease, prostatic cancer and male sterility. We are focusing on the study of Katanin in C. elegans, which comprises a catalytic AAA+ subunit (p60 --MEI-1 in C. elegans) and a regulatory subunit (p80-like --MEI-2 in C. elegans). Katanin is essential for meiotic spindle assembly but the relative contribution of the MEI-1 and MEI-2 subunits is still incompletely understood. We have shown previously that the Katanin microtubule-severing activity, but not the microtubule-bundling activity, is essential for female meiotic spindle assembly. Indeed, separation-of-function mei-1 mutants that retain the MT-bundling activity but are defective in MT-severing fail to assemble a meiotic spindle. Furthermore, we demonstrated that the regulatory MEI-2 subunit directly interacts with microtubules. Using a combination of genetic and biochemical approaches, we are currently trying to decipher the mechanism by which Katanin severs microtubules. Reference: Joly N, Martino L, Gigant E, Dumont J, Pintard L. Microtubule-severing activity of AAA-ATPase Katanin is essential for female meiotic spindle assembly. Development. 2016 Oct 1;143(19):3604-3614.

P-137 Germline-specific protein interaction network of the germline fate-protecting chromodomain protein MRG-1

Gülkiz Baytek[1, 2], Alexander Gosdschan[1, 3], Martina Hajduskova[1], Marlon Kazmierczak[1, 3], Philipp Mertins[2], Baris Tursun[1]

[1]*Berlin Institute for Medical Systems Biology, Max Delbrück Center, Berlin, Germany*

[2]*Proteomics Core Facility, Max Delbrück Center, Berlin, Germany*

[3]*Department of Biology, Humboldt University, 10115 Berlin, Germany*

Direct reprogramming of cellular identities by ectopically-expressed transcription factors (TFs) is limited in different tissue contexts. To identify reprogramming barriers in C. elegans, we performed a whole-genome RNAi screen (Kolundzic et al. 2018) by making use of transgenic animals that broadly express the ASE neuron fate-inducing TF CHE-1. Several factors have been identified including LIN-53 (Tursun et al., 2011), the FACT (Kolundzic et al. 2018) complex and MRG-1 that safeguard germ cells against germ cell conversion. Given that these chromatin regulators are known to function in complexes, interrogating their protein partners is key to elucidate the underlying mechanisms of how they safeguard a specific cell fate. First, we optimized a native Co-Immunoprecipitation (co-IP) protocol by adopting Single-Pot Solid-Phase-enhanced Sample Preparation (SP3) to

worm lysates, which is followed by mass spectrometry for C. elegans lysates. With this procedure we were able to identify novel interactors of the chromodomain-containing protein MRG-1, whose functions at the molecular level in C. elegans have not been deeply studied but, instead, annotated from its mammalian homolog MRG15. Although MRG-1 is broadly expressed in C. elegans, its knockdown by RNAi resulted in reprogramming solely in the germline. In order to elucidate why this conversion is restricted to the germline, we made use of a temperature sensitive glp-4 (bn2) mutant, which does not develop a germline under restrictive temperature conditions. By comparing the IP data for wild-type and glp-4 mutant animals, we identified the germline-specific interaction network of MRG-1. Moreover, our results indicate that MRG-1, together with the chromatin-regulating protein SET-26, blocks the conversion of germ cells to ASE neurons. Overall, the interaction network of MRG-1 comprises proteins of different classes including RNAi-binding proteins that have been in implicated in chromatin regulation and RNA processing.

P-139 LITE-1 AS AN OPTOGENETIC TOOL - CHARACTERIZATION AND SIGNAL-PATHWAY

Marcial Alexander Engel[1, 2], Franziska Hannig[1, 2], Dana Maureen Hebchen[1, 2], Bojana Languille[1, 2], Christina Schüler[1, 2], Alexander Gottschalk[1, 2]

[1]*Buchmann Institute for Molecular Life Sciences, Goethe University, Max von Laue Strasse 15, D-60438 Frankfurt, Germany*

[2]*Institute of Biophysical Chemistry, Goethe University, Max von Laue Strasse 15, D-60438 Frankfurt, Germany*

LITE-1 was identified as the major photoreceptor responsible for the light response in *C. elegans*. LITE-1 is localized in neurons in the worm's head and tail where it recognizes short-wavelength light and evokes negative phototaxis. Ectopic expression of LITE-1 in *C. elegans* under the *pmyo-3* promoter in body wall muscle (BWM) cells enables the excitation of the naturally non-photosensitive cells. It causes a long-lasting blue light induced muscle contraction, that can be measured as a reduction of body length. This suggests LITE-1 as an interesting new optogenetic tool. In contrast to rhodopsins no associated chromophore has been identified yet, but tryptophan residues might be involved in light absorption. However, the signaling pathway evoked by this light stimulation differs from the one in neuronal cells, since muscle cells lack cyclic nucleotide-gated (CNG) channels and guanylyl cyclases. To understand the signaling pathway of LITE-1 in BWMs, we are using RNA interference and/or mutants of several putative members of the LITE-1 signaling pathway in BWM and tested them for a reduction of light triggered muscle contraction. We found that the alpha-1 subunit of L-type voltage-gated Ca^{2+} channel $Ca_V1.2$ (encoded by *egl-19*) seems to be responsible for LITE-1 evoked muscle contraction. Also the knockdown of several Gα proteins led to reduced LITE-1 evoked muscle contraction, which hints for two different pathways. Currently, we are investigating further steps of the signal transduction. Elucidating the pathway may allow establishing LITE-1 as a novel optogenetic tool.

P-141 Active RHO-1 forms clusters at the equatorial cortex during cytokinesis.

Jennifer Sacher, Esther Zanin

Center for Integrated Protein Science, Department Biology II, Ludwig-Maximilians University Munich, Planegg-Martinsried, Germany

Cytokinesis is the last step of cell division during which the mother cell is physically divided into two daughter cells. Failure of cytokinesis results in tetraploidy and supernumerary centrosomes that can cause oncogenic transformation. Constriction of the mother cell is mediated by a contractile ring that assembles underneath the plasma membrane during anaphase. Formation of the contractile ring is triggered by the activation of the small GTPase RHO-1 (RhoA in humans), which in turn induces actin polymerization and myosin II activation. RHO-1 is activated in a narrow zone at the equatorial cortex by the guanine nucleotide exchange factor (GEF) ECT-2. How active RHO-1 rapidly accumulates and is maintained within this narrow zone is an important question in the field of cell division. To investigate RHO-1 dynamics during cytokinesis we established a functional RNAi-resistant GFP-tagged RHO-1 transgene. Live-cell imaging of the cell cortex, in the absence of endogenous RHO-1, revealed that GFP::RHO-1 is enriched in clusters at the anterior and equatorial plasma membrane. To test whether RHO-1 clusters contain active RHO-1 we depleted ECT-2. While anterior RHO-1 clusters were still formed, equatorial

clusters were absent in ECT-2 depleted embryos, suggesting that they contain active RHO-1. To investigate how membrane binding and clustering of active RHO-1 is controlled, we analyzed the contribution of two putative membrane targeting motifs of RHO-1: the poly-basic sequence (PBS) and the CAAX motif. Our data suggest that both the PBS and CAAX motif are required for RHO-1 membrane binding and function. However, they are not sufficient to target RHO-1 to equatorial RHO-1 clusters. In summary, active RHO-1 forms clusters at the equatorial cortex during cytokinesis. We hypothesize that clustering of active RHO-1 maintains and stabilizes active RHO-1 at the site for furrow formation and thereby facilitates contractile ring assembly and successful cytokinesis.

P-145 New roles for microtubules in zygote polarity

Jack Adam Martin, Josana Rodriguez

Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle upon Tyne NE2 4HH, UK

Zygote polarity relies on the asymmetric distribution of key polarity effectors, the PAR proteins, which in turn drive the zygotes' asymmetric cell division leading to the germline and somatic cell precursors. Zygote polarisation is triggered by the sperm-donated centrosome via two semi-redundant pathways. First, the centrosome reorganises the cortical acto-myosin meshwork, inducing a cortical flow away from the newly defined posterior pole. This flow transports a subset of PAR proteins to the anterior half of the zygote. Second, centrosomal-microtubules (MT) induce the membrane loading of another set of PAR proteins at the posterior. Anteriorly and posteriorly localised PARs mutually antagonise each other, further ensuring their asymmetric distribution. The existence of these two pathways confers robustness, but also makes it hard to tease these mechanisms apart and has impede the identification of regulatory components for the MT pathway. We reasoned that knock-down of MT-pathway regulators in a mutant strain where the acto-myosin flow is perturbed, should lead to strong polarity defects and lethality that are not observed when knocked-down in a wild-type strain. Using this strategy we have identified MT-pathway candidates and their characterisation is starting to reveal new roles for microtubules in zygote polarity.

P-147 Generation & implementation of membrane-bound adenylyl cyclases as optogenetic tools with CNG-gated ion channels in Caenorhabditis elegans

Thilo Henß[1, 2], Jatin Nagpal[3], Shiqiang Gao[4], Ulrike Scheib[5], Alexander Hirschhäuser[1, 2], Franziska Schneider-Warme[6], Peter Hegemann[5], Georg Nagel[4], Alexander Gottschalk[1, 2]

[1]Buchmann Institut for Molecular Life Sciences (BMLS), Goethe University, Max von Laue Str. 15, 60438 Frankfurt, Germany

[2]Institute for Biophysical Chemistry, Goethe University, Max von Laue Str. 15, 60438 Frankfurt, Germany

[3]Deutsches Resilienz Zentrum, Johannes Gutenberg University Medical Center, Langenbeckstr 1, 55131 Mainz, Germany

[4]Department of Biology, Institute for Molecular Plant Physiology and Biophysics, Biocenter, Julius-Maximilians-University of Würzburg, Julius-von-Sachs-Platz 2, D-97082 Würzburg, Germany

[5]Humboldt University Berlin, Institute of Biology, Experimental Biophysics, Invalidenstraße 42, D-10115 Berlin

[6]Institut für Kardiovaskuläre Medizin, Elsässer Straße 2Q, 79110 Freiburg im Breisgau, Germany

In eukaryotic GPCR signaling, adenylyl cyclases (ACs) acting downstream of Gαs are plasma membrane-bound and are located in signalosomes together with GPCRs, protein kinase A (PKA) and their targets. To date, the only existing optogenetic tools for cyclic adenosine monophosphate (cAMP) generation are soluble proteins (e.g. Beggiatoa or Euglena photoactivated ACs - bPAC or EuPAC). To enable more specific optogenetic cAMP signaling, membrane-bound photoactivated ACs (mb-PACs) were generated and implemented as optogenetic tools in cholinergic motor neurons and body wall muscle cells of Caenorhabditis elegans. The membrane-bound ACs were either generated by adding membrane tethers, or by mutating the guanylyl cyclase domains from Blastocladiella (Be) and Catenaria (Ca) cyclase opsins (CyclOps), which combine a light-absorbing rhodopsin domain and a highly specific guanylyl cyclase domain into a single molecule. To conclude on the amount of generated cAMP, the impact of the mb-PACs on C. elegans behaviour upon illumination was analyzed. Expressing the proteins in cholinergic motor neurons, the mb-PACs were assessed for their potential to increase C. elegans locomotion

behavior, which is enhanced by cAMP generation. Co-expressing the proteins with cyclic nucleotide gated (CNG) cation channels (conducting either Na+ or K+) in body wall muscle cells, the mb-PACs were analyzed for their potential to reduce or elongate the C. elegans body length (as a proxy for de- or hyperpolarization, respectively). Among the tested ion channels were TAX-2/TAX-4, an unspecific cation channel, whose activation leads to the depolarization of the muscle cells. This channel is mainly cGMP-gated, however, at high concentrations it responds also to cAMP. In addition, we tested CNG channels that are K+-channels gated by cGMP or by cAMP, whose activation causes hyperpolarization of muscle cells.

P-149 Investigating in vivo variation in the strength of the spindle assembly checkpoint

Abigail Gerhold[1, 3], Vincent Poupart[1], Paul Maddox[2], Jean-Claude Labbé[1]

[1]Institute for Research in Immunology and Cancer, Université de Montréal, Montreal, Quebec

[2]Department of Biology, University of North Carolina, Chapel Hill, Chapel Hill, North Carolina

[3]Department of Biology, McGill University, Montreal, Quebec

The spindle assembly checkpoint (SAC) is a conserved mitotic regulator that preserves genome stability. Despite its central role in maintaining mitotic fidelity, variation in SAC strength is a widespread, but poorly understood, feature of checkpoint regulation. Notably, early embryonic cells generally have a weak checkpoint, suggesting developmental input. Using in situ live imaging, we have shown that C. elegans germline stem cells (GSCs) have a stronger SAC than somatic blastomeres, providing an excellent opportunity to examine variability in SAC activity during development in vivo. Here we show that the embryonic progenitors of GSCs also display a stronger SAC, relative to their somatic peers. These differences are entirely dependent on an intact checkpoint and only partially attributable to differences in cell size. In 2-cell embryos, cell size accounts for half of the difference in SAC strength between the larger somatic AB and the smaller germline P1 blastomeres. The remaining difference requires asymmetric cytoplasmic partitioning downstream of PAR polarity proteins, suggesting that checkpoint regulating factors are distributed asymmetrically during early germ cell divisions. Our results indicate that germline-fated cells have a stronger SAC, suggesting that varying SAC strength may be adaptive, and reveal a novel interaction between asymmetric cell division and the SAC.

P-151 A novel tumor related gene, HPO-11/NRBP affects mRNA fate in C. elegans

Qian Zhao[1], Wenjing Qi[1], Ralf Baumeister[1, 2]

[1]Bioinformatics and Molecular Genetics (Faculty of Biology), Albert-Ludwigs-University of Freiburg, Freiburg, Germany

[2]ZBMZ (Faculty of Medicine), Albert-Ludwigs-University of Freiburg, Freiburg, Germany

Modulation of mRNA fate is one of the key aspects of posttranscriptional control of eukaryotic gene expression. In coping with various stressors, eukaryotic cells form several types of cytoplasmic, non-membrane bound ribonucleoprotein (RNP) granules, including stress granules (SGs) and processing bodies (PBs), to re-establish cellular homeostasis under adverse conditions. SGs contain translational stalled mRNA, translational initiation factors and additional proteins affecting mRNA function while PBs contain mRNA, machineries for mRNA decay or translational repression. Cytoplasmic mRNAs cycle between SGs and PBs. In addition, SGs and PBs physically interact and are often docking each other in mammalian cells during stress. However, mechanisms directing mRNA movement between SGs and PBs are not known yet. In our previous study, our lab has identified involvement of the hpo-11 gene, homologue of Drosophila MADM/Mlf1 and the human tumor suppressor NRBP1, in germline tumor formation in C. elegans. HPO-11/NRBP1/MADM are pseudo-kinases, and probably have an adaptor-like function in mediating protein-protein interaction. We observe that HPO-11 is typically uniformly distributed in cells, but localizes in cytoplasmic foci under different stress conditions (heat, osmotic and oxidative stress). Co-localization analysis indicated that these cytoplasmic foci are the SGs and PBs. In addition, hpo-11 knock-down does not prevent SGs or PBs formation, but significantly reduces SGs localization of the translation initiation factor IFE-2/eIF4E, suggesting that HPO-11 may affect the composition of SGs. Furthermore, subcellular distribution of translational initiation factor IFG-1/eIF4G is also altered upon hpo-11 knockdown under stress condition. As eIF4E together with eIF4G binds to 5'cap of mRNA, we are currently

addressing whether HPO-11 affects fate of mRNA under stress conditions, focusing on the dynamic localization of components of the translational machinery into SGs or PBs.

P-153 Heparan sulfate proteoglycans roles in morphogenesis and nervous system development in *C. elegans*

Marianne Moore[1], Lise Rivollet[1], Dan Shaye[2, 3], Claire Bénard[1, 3]

[1]*Department of Biological Sciences, BioMed Research Center, Université du Québec à Montréal, Canada*

[2]*Department of Physiology and Biophysics, University of Illinois - Chicago, USA*

[3]*Co-senior Authors*

The regulation of cell shape and migration is essential to animal development and physiology. Heparan sulfate proteoglycans regulate interactions of morphogens and guidance cues with their respective receptors to elicit appropriate cellular responses. Heparan sulfate proteoglycans consist of a protein core with attached heparan sulfate glycosaminoglycan chains, which are synthesized by glycosyltransferases of the exostosin (EXT) family. Abnormal HS chain synthesis results in pleiotropic consequences, including abnormal development and tumor formation. Complete loss of any of the exostosin glycosyltransferases in mouse, fish, flies and worms leads to drastic morphogenetic defects and embryonic lethality. We have identified viable hypomorphic mutations in the two *C. elegans* exostosin glycosyltransferases genes, *rib-1* and *rib-2*, which lead to a severe reduction of HS levels and result in profound but specific developmental defects, including abnormal cell migrations and the occurrence of supernumerary cellular projections in neurons and the excretory canal cell. We are addressing how HSPGs regulate cell shape and morphology, in particular, how they keep in check the number of projections of polarized cells.

P-155 Exploring the functional conservation of a deeply conserved animal microRNA

Paula Gutiérrez Pérez, Anna Schrempf, Luisa Cochella

Research Institute of Molecular Pathology (IMP), Vienna, Austria.

miR-1 is one of the most conserved microRNAs from C. elegans to humans not only in terms of sequence, but also in terms of its specific expression in muscle, and its function in muscle integrity and development. Moreover, miR-1 is the only microRNA whose predicted targets show a remarkable level of conservation across evolution. From C. elegans to human, miR-1 has predicted binding sites in the 3'UTRs of multiple subunits of the vacuolar-ATPase (V-ATPase) complex. However, no experimental evidence supporting this predicted interaction has been reported. On the contrary, only phylogenetically unrelated targets have been implicated in miR-1 function, in the different animal models. Here, we set out to address whether miR-1 has a functional relationship with the V-ATPase complex that could account for its conservation. In higher organisms, loss of miR-1 causes lethality due to heart problems. However, in C. elegans, miR-1 deficient animals are viable. Together with the powerful genetic tools available to study miRNA function, this makes this animal a great model to uncover this relationship. We have analyzed the expression pattern of different subunits of the V-ATPase complex in miR-1 mutant animals compared to wild-type ones. We have found an upregulation of vha-1 and -12 in muscle cells after the depletion of the miRNA. In addition, we have identified a subtle morphological and functional defect in miR-1 mutant animals. These animals are longer than wild-type worms and display a defect in pharyngeal pumping: longer pump duration and consequently, lower pumping frequency. These results uncover a role for miR-1 in pharyngeal muscle and provide a functional readout that we can use for further genetic studies. In the longer term, by analyzing the link between the V-ATPase complex and miR-1 in other species, we expect to find conserved pathways required for the establishment of muscle identity and function.

P-157 Oligomerization of the RZZ (Rod-Zwilch-Zw10) complex drives expansion of the kinetochore corona

Cláudia Pereira[1, 2], Rita Reis[1, 2], José Gama[1, 2], Dhanya Cheerambathur[3], Ana Carvalho[1, 2], Reto Gassmann[1, 2]

[1]*Instituto de Biologia Molecular e Celular, Universidade do Porto, 4200-135 Porto, Portugal*
[2]*Instituto de Investigação e Inovação em Saúde (I3S), Universidade do Porto, 4200- 135 Porto, Portugal*
[3]*Ludwig Institute for Cancer Research/Department of Cellular and Molecular Medicine, University of California at San Diego, La Jolla, CA 92093, USA*
The kinetochore is a dynamic multi-protein assembly that forms on each sister chromatid and interacts with microtubules of the mitotic spindle to drive chromosome segregation. In animals, kinetochores without attached microtubules expand their outermost layer into crescent and ring shapes to promote microtubule capture and spindle assembly checkpoint (SAC) signalling. Kinetochore expansion is an example of protein co-polymerization, but the mechanism is not understood. We have observed that the ROD-ZWILCH-ZW10 (RZZ) complex is essential for kinetochore expansion in cultured human cells. Moreover, recent analysis of reconstituted human RZZ by cryo-electron microscopy showed that the ROD subunit is structurally related to membrane coat proteins such as Clathrin and subunits of the COPI and COPII complexes, that form higher-order assemblies around vesicles. However, evidence that ROD proteins are capable of self-assembly is currently missing. Using the C. elegans early embryo, we demonstrate that ROD-1 has a concentration-dependent propensity for oligomerizing into µm-scale filaments, and we identify the ROD-1 β-propeller as a key regulator of self-assembly. In vitro, we show that a minimal ROD-1-ZW10 complex efficiently oligomerizes into filaments. Our results support the idea that RZZ's capacity for oligomerization is harnessed by kinetochores to assemble the expanded outermost domain, in which RZZ filaments serve as recruitment platforms for SAC components and microtubule-binding proteins. Thus, we propose a model where RZZ self-assembly into filaments underlies the adaptive change in kinetochore size that contributes to chromosome segregation fidelity.

P-159 Molecular mechanisms of synaptic tiling

Kota Mizumoto
Department of Zoology, University of British Columbia
During development, neurons form extensive synaptic connectivity with their fate-determined targets. Ectopic synapse formation with aberrant targets underlies various neurological disorders including autism. While we are beginning to elucidate the underlying mechanisms by which extracellular ligand-receptor interactions enhance synapse specificity by inhibiting synaptogenesis, our knowledge about their intracellular mechanisms remains limited. Here we show that Rap2 GTPase (rap-2) and its effector, TNIK (mig-15), act downstream of Plexin (plx-1) signaling to restrict presynaptic assembly and to form tiled synaptic innervation of two cholinergic motor neurons (DA8 and DA9) in C. elegans. Both constitutively GTP- and GDP-forms of rap-2 mutants exhibit similar synaptic tiling defects as plx-1 mutants, suggesting that cycling of the RAP-2 nucleotide state is critical for synapse inhibition. Consistently, RAP-2 activity is suppressed in a short segment of axon lacking synapses where PLX-1 is enriched. Excessive ectopic synapse formation in mig-15 mutants causes expansion of the synaptic domains in DA8 and DA9, which induces a severe synaptic tiling defect. Conversely, overexpression of mig-15 strongly inhibited synapse formation, which suggests that mig-15 is a negative regulator of synapse formation. These results reveal that subcellular regulation of small GTPase activity by Plexin shapes proper synapse patterning in vivo. We will discuss additional mechanisms of synaptic tiling at the meeting.

P-161 Developmental regulation of germline syncytium organization in C. elegans

Jack Bauer[1], Mei Zhen[2], Jean-Claude Labbé[1]
[1]*Institute of Research for Cancerology and Immunology (IRIC)*
[2]*Lunenfeld-Tanenbaum Research Institute, 600 University Ave, Toronto, ON M5G 1X5, Canda*
While a syncytial architecture is common among animal germlines and is required for fertility, the mechanisms leading to syncytium formation during development are largely unknown. The nematode C. elegans constitutes a powerful in vivo model to study syncytium formation and organization throughout development. In the embryo, incomplete division of the germline precursor blastomere P4 gives rise to the two primordial germ cells (PGCs) that remain stably interconnected by a cytoplasmic bridge enriched in conserved contractility regulators. In the larval and adult germline, these contractility regulators are found at the stable actomyosin rings that connect

germ cells to the central rachis. We are characterizing the structure of the two PGCs at the L1 larval stage to understand the relationship between the stable cytoplasmic bridge that forms between the PGCs during embryogenesis and the syncytial germline architecture of adult animals. Our preliminary results reveal the presence of a syncytial structure between the two PGCs of L1 stage animals, organizing into a proto-rachis in which contractility regulators form actomyosin rings that interconnect the PGCs and several membrane lobes to a common, central cavity. To understand how cells remain connected to the proto-rachis after division, we track the first division of the PGCs to characterize the ingression of the cytokinetic ring. Investigating the developmental regulation of C. elegans PGCs will provide a better understanding of the mechanisms required for germline syncytium formation and organization.

P-163 COPAS Vision is new instrumentation that can take pictures and sort worms

Rock Pulak, Julia Thompson, Tom Mullins, Mariya Lomakina, Bruce Holcombe, Mikalai Malinouski, Chris Bogan
Union Biometrica, Inc

From their beginning the original COPAS instruments were designed to work with C. elegans and soon got a nickname “the worm sorter”. Based on the same principles as traditional flow cytometers, worms are suspended in liquid, sent through a flow cell, pass through a laser and a few measurements are taken. These are size (TOF), optical density (EXT), and fluorescence from three regions of the spectrum, usually green, yellow, and red. Then, if the measurements indicate it’s a worm you want, that worm can be sorted out of the stream and collected on a plate, or to a well of a multiwell plate, or some other receptacle of ones choosing. When we first started making COPAS instruments we often thought it would be helpful if we could take pictures of the worms as they travel through the flow cell. This is now possible. The COPAS Vision is a new instrument that can take a brightfield image of the worms. No image analysis occurs live but image collection is synchronized with the flow cytometry data and that flow cytometry data can be used to make a sorting decision. The instrument can collect and store images for every worm in the sample or only those that meet a certain criteria, such as size or combination of colors. A few other changes were made, like more lasers can be added to this COPAS (up to 4) and more fluorescent colors can be detected (up to 8). We will present C. elegans data collected from the COPAS Vision that shows some of the capabilities of this new instrumentation.

P-165 Exploring the role of the ULP-2 SUMO protease in the germline

Ulrike Bening, Marana Abboud, Limor Broday
Department of Cell and Developmental Biology, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel

The ubiquitin-like SUMO system is essential for cell function, acting mainly through reversible regulation of protein-protein interactions. SUMO modification is highly dynamic and its deconjugation is regulated by specific cysteine proteases that cleave the isopeptide bond between the SUMO moiety and substrates. We have previously showed that the SUMO protease ULP-2 is required for embryonic morphogenesis and that HMR-1 is a key target of ULP-2 deconjugation activity during this developmental stage. Using the CRISPR/Cas9 approach we have generated a predicted null allele of ulp-2, ulp-2(tv380). Homozygous mothers of this allele are fertile at 20°C but the majority of progeny are arrested during epidermal morphogenesis (63.4% embryonic arrest). Surprisingly, the remaining embryos that succeed to complete epidermal morphogenesis continue to develop till adulthood. These adults are completely sterile with abnormal somatic gonads and germline. The germline appear relatively healthy at the L4 stage but deteriorate in adults. DAPI staining revealed smaller gonads with fewer cells than WT and the proximal region containing non- or only few oocytes in diakinesis. To examine if the germ cells were reprogrammed to somatic fate we analyzed unc-119::GFP expression and indeed detected expression of this reporter in the proximal gonad arms in cells with neurite-like projections. In a proteomics screen for ULP-2 associated proteins, we identified ASH-2, the ortholog of the Drosophila absent, small, or homeotic discs 2 (Ash2). Ash2 is a component of H3K4 HMTase complex and a member of the trithorax family. In C. elegans, ASH-2 was shown to contribute to H3K4 methylation. RNAi of ash-2 enhance the sterility phenotype of the ulp-2(tv380) allele, resulting in complete sterility in the F1 homozygous animals. Our observations suggest that ULP-2 is

required for regulation of H3K4 HMTase activity in the germline to protect germline specification and proliferation.

P-167 Suppressor analysis reveals new genes involved in embryonic cell migration

Vida Praitis, Zoe Scott-Nevros, Haonan Sun, Shaina Zarkin-Scott

Grinnell College Biology, Grinnell IA USA

The C. elegans embryo is an excellent model for studying cell migration in three dimensional systems. During development, cell migrations, including ingression, cell rearrangements following ingression, dorsal intercalation, and ventral closure, utilize distinct and likely conserved genetic pathways. Our laboratory showed pmr-1/SPCA, a secretory store calcium ATPAse, is required for cell rearrangements that follow ingression. pmr-1 loss-of-function mutants die during embryogenesis due to cell migration defects. We reasoned other gene products involved in calcium homeostasis and signaling play roles during cell migration, but these may not have been identified because of redundancy or pleiotropy. To identify these genes, we used pmr-1 conditional alleles to perform both candidate RNAi and forward genetic suppressor screens. The forward screen identified strains carrying suppressors of the pmr-1(ru5) lethality at 25C and inheritance of suppression is maternal. Suppressor strains also show associated phenotypes, such as hermaphrodite sterility and male mating defects. To identify the altered genes, we performed both snp-snp mapping and whole genome mapping and sequence analysis. Preliminary analysis reveals candidates that have calcium-related functions, including one included in the candidate RNAi screen. We plan to discuss suppressor candidates at the meeting. The feeding RNAi screen also identified suppressors. RNAi of candidate genes in pmr-1(ru5) tended to have weaker improvements in viability than those identified in the forward screen, perhaps because disruptions in gene dosage of these candidates need to be precisely balanced by disruptions of pmr-1. RNAi of the proprotein convertase genes bli-4, egl-3, or kpc-1 suppresses the lethality of pmr-1(ru5) at 20C, but only egl-3 does so significantly at 25C. In a pmr-1(+) background, disruption of kpc-1 and bli-4, but not egl-3, also causes embryonic lethality. These findings suggest that proprotein convertases act during cell migration in opposition to PMR-1, and that their roles in this process are likely masked by redundancy.

P-169 Analysis of the transcriptional basis of natural transdifferentiation in C. elegans

Jaime Osuna Luque[1, 2], Peter Meister[1], Sophie Jarriault[2]

[1]*Cell Fate and Nuclear Organization, Institute für Zellbiologie, University of Bern.*

[2]*In vivo cellular plasticity and direct reprogramming, IGBMC, University of Strasbourg, CNRS UMR7104, INSERM U1258.*

We are studying a natural transdifferentiation event naturally occurring in vivo in a single cell in 100% of the animals with 100% efficiency. This cell transdifferentiates from a rectal fate (Y cell fate) into a moto-neuron identity (PDA cell fate). This system has contributed key insights on the transition and cellular steps involved and the identification of conserved nuclear factors crucial to the initiation of the process, or the relative importance and roles of transcription factors versus histone modifying factors for the dynamics and robustness of the conversion. The exact transcriptional dynamics of these genes remains unknown. If the transdifferentiation process is highly efficient, it remains challenging to be modelled in vitro as no cell culture and cell lines exist and as cell can alter their expression programmes whan cultured in exogenous conditions. We therefore need to examine the transcriptional dynamics of a single cell in vivo in entire animals. To achieve this, we are developing an RNA polymerase II footprinting technique, based on DNA adenine methyltransferases identification (DamID). DamID makes use of a fusion protein between RNA polymerase subunits and a bacterial DNA methylase (Dam). Binding of the RNA polymerase to transcribed genes leads to their DNA methylation, which allows the subsequent identification of these genes using molecular techniques. To restrict expression of the Dam fusion to the transdifferentiating cell (Y/PDA), we combine two recombination systems (FRT/FLP and Cre/lox). We temporally control the onset of the footprint during development (in the Y cell, during the transition or or in the PDA neuron) using an auxin-induced degradation system, in which an externally applied plant hormone leads to protein degradation. These technical developments of DamID will be presented. Once the footprint has been

determined, we will further validate our results using smFISH. The functional role during transdifferentiation of genes turned on or off will then be examined through and knock-out/down and functional experiments.

P-171 A genetic screen for morphogenesis-defective, temperature-sensitive mutants in Caenorhabditis elegans

Molly Jud[1], Josh Lowry[1], Thalia Padilla[1], Erin Clifford[1], Yuqi Yang[1], Alexander Miller[1], Hong Shao[2], Nhah Tran[2], Zhirong Bao[2], Bruce Bowerman[1]

[1]*Institute of Molecular Biology, University of Oregon, Eugene, OR*

[2]*Developmental Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY*

Morphogenesis comprises the coordinated migrations and shape changes of cells to form organs and body plans during embryonic development. Cell adhesion and the cytoskeleton have known roles during morphogenesis; however, the signaling and biochemical pathways involved remain incompletely understood. Since genes that regulate morphogenesis often have requirements earlier in development, temperature-sensitive, embryonic lethal (TS-EL) alleles provide a useful tool for identifying and investigating genetic pathways necessary for morphogenesis. The Bowerman lab has isolated a collection of ~1,000 TS-EL mutants, and we seek to identify and clone all the morphogenesis-defective mutants in this collection. We have identified 109 with terminal elongation-defective phenotypes (78 penetrant and 31 variable mutants arresting at a single embryonic stage ≥70% or 50<70% of the time, respectively), with the majority arresting without elongation. We identify the causative genes using a combined SNP mapping and whole genome sequencing approach, along with genetic complementation tests. So far, we have identified 22 alleles representing 16 genes, including glp-1 (3 alleles), let-19 (3 alleles), emb-5 (3 alleles), mom-4, nap-1, gad-1, emb-4, chaf-1, fntb-1, rib-1, hlh-1, sart-3, cdc-25.2, and lrr-1. The mutants we identify are further analyzed using an automated cell lineaging platform to distinguish between mutants exhibiting cell fate specification versus morphogenesis defects. Many of the genes identified have roles in cell fate specification. In an effort to identify mutants that are specifically defective in morphogenesis, we are now shifting prebean and bean staged embryos from the permissive to the restrictive temperature. Mutants in three genes (rib-1, fntb-1, and hlh-1) have penetrant elongation-defective phenotypes with bean stage upshifts. Our long term goal is to advance the understanding of embryonic morphogenesis by identifying previously unknown players influencing this fundamental biological process.

P-173 Genetic basis of natural variation in X chromosome nondisjunction in C. elegans

Jun Kim, Jiseon Lim, Yeeun Yoon, Junho Lee

School of Biological Sciences, Seoul National University, Seoul 08826, Republic of Korea

Cross-fertilization increases genetic diversity by mixing genomes of two different individuals, so that the outcrossing can facilitate rapid adaptation to a novel environment. However, C. elegans uses a different reproductive strategy. This species reproduces primarily by self-fertilization of hermaphrodites, thereby producing ~99.9% of hermaphrodites again. Due to low presence of male worms, outcrossing is limited in natural population. The rare proportion of male offspring comes from low rate of X chromosome nondisjunction in hermaphrodites. Although from a limited survey, the nondisjunction rate has been suggested to show natural variation across wild isolates of C. elegans (Teotónio, H., Manoel, D., & Phillips, P. C., 2006). In this study, we sought to find causal genes that generate the natural variation and to identify whether or how the genes affect outcrossing and adaptation. We used 95 wild isolates from the C. elegans natural diversity resource (CeNDR) (Cook, D. E., et al., 2016) and measured proportion of male offspring produced by virgin hermaphrodites to identify natural variation in male proportion, brood size, and age-dependent male production. We identified three quantitative trait loci (QTLs) of male proportion. We will present updated results of the examination of near isogenic lines (NILs) for the candidate region created by introgressing a high male proportion strain into a low male proportion strain.

P-175 Regulation of ERM proteins in cortical membrane specialization

Joao Ramalho, Mike Boxem

Developmental Biology Utrecht University

The establishment of specialized cortical domains is central to the functioning of polarized cells and epithelial tissues. Cortical specialization requires coordinated remodeling of the plasma membrane and the underlying cytoskeletal actin network. Proteins of the conserved Ezrin-Radixin-Moesin (ERM) family interact with membrane lipids and proteins through an N-terminal FERM domain, as well as with F-actin via the C-terminal domain. In different model systems, ERM linker activity has been associated with formation of specialized structures such as microvilli, axonal growth cones, or the leading edge of migratory cells. Activity of ERM proteins depends on a conformational change that turns an inactive cytoplasmic form into an active membrane- and actin-bound form. In vitro data suggests a two-step activation model in which PIP2-binding is followed by phosphorylation of a conserved C-terminal threonine residue. However, in vivo data supporting this model is scarce and contradictory. We use the single C. elegans ERM ortholog, ERM-1, as a model to study the contribution of different regulatory sites for ERM protein activity and tissue morphogenesis in vivo. Using CRISPR/Cas9 editing to generate different erm-1 mutant alleles we show that PIP2-binding, but not C-terminal T544 phosphorylation, is critically required for ERM-1 function. erm-1 mutants unable to bind PIP2 mimic the null phenotype. In contrast, mutants that either constitutively lack or mimic T544 phosphorylation are viable, with defects whose severity differs between tissues. Our results indicate that dynamic regulation of T544 phosphorylation status and phosphocycling is required for proper epithelial lumen formation and modulates ERM-1 localization, stability, and activity in a tissue-specific manner. Elucidation of the mechanisms that regulate ERM protein function will contribute to our understanding of cortical remodeling events in development and disease.

P-177 Molecular mechanisms of developmentally controlled polyploidization in the C. elegans intestine

Lotte van Rijnberk[1], David Morgan[2], Matilde Galli[1]

[1]*Hubrecht Institute, Utrecht, Uppsalalaan 8, The Netherlands*

[2]*Department of Physiology and Biochemisty & Biophysics, University of California, San Francisco, San Francisco, CA 94143, USA*

Polyploid cells, which contain more than two homologous sets of chromosomes, can arise in certain tissues as part of a developmental program, where they are critical for increases in cellular output and mass. Two cell cycle variations are known to generate polyploid cells: endoreplication and endomitosis. During endoreplication, cells skip M phase completely, whereas endomitotic cells enter M phase but prematurely exit mitosis and do not complete cytokinesis. Although the molecular regulation of endoreplication has been studied extensively, the molecular factors that control endomitosis remain largely unknown. Specifically, it is unclear how endomitotic cycles are initiated and executed during development. To study this, we use the C. elegans intestine, which transitions between canonical, endomitotic and endoreplicative cycles at known moments during development. Our analysis of mitotic spindle morphology revealed that endomitotic cells fail to form a central spindle during anaphase. Since several protein complexes involved in the formation of the central spindle are also essential for cytokinesis, we hypothesized that the downregulation of one of these complexes could explain the absence of cytokinesis during endomitosis. Strikingly, we found that both members of the centralspindlin complex, ZEN-4 and CYK-4, are absent during endomitosis. Our single molecule FISH analyses of endomitotic cells revealed that zen-4, cyk-4 and another cytokinesis regulator, spd-1 (Prc1), are all downregulated during intestinal endomitosis compared to other larval divisions. In contrast, the general mitotic gene cyb-1 is similarly expressed during endomitosis and canonical cycles, suggesting that endomitotic cells specifically downregulate cytokinesis genes. We are currently trying to identify which upstream factors are required to set up an endomitotic program by performing tissue-specific RNA sequencing and RNA Tomography sequencing (Tomo-seq) of intestinal cells at different cell-cycle stages. Together this will give an in-depth analysis of endomitosis, providing insights into how cell-type specific variations in cell cycles arise during development.

P-179 Second male meiotic division is independent on the canonical spindle assembly checkpoint signaling in C. elegans

Shang-Yang Chen[1], Jui-ching Wu[1, 2]

[1]*Department of Clinical Laboratory Sciences and Medical Biotechnology College of Medicine, National Taiwan University*

[2]*Department of Laboratory Medicine, National Taiwan University Hospital, Taipei, Taiwan*

Spindle assembly checkpoint (SAC) safeguards the fidelity of chromosome segregation during cell division. During meiosis, duplicated chromosomes undergo two consecutive chromosome separation events. It is unclear whether both chromosome segregation events are regulated by SAC. We found outer kinetochore protein BUB-1 is recruited back to chromosomes after first male meiotic division, suggesting the kinetochore is re-established between two divisions. To test if SAC signaling is also reformed after meiosis I, we examined the dynamics of SAC target securin IFY-1. As expected, IFY-1 levels drop drastically at first division. To our surprise, the levels of IFY-1 did not regained and remained absent from second male meiotic division. This suggests proteasome-dependent securin degradation is not the main regulation target for meiosis II. Consistent of this, treatment of proteasome inhibitors failed to stop chromosome segregation in secondary spermatocytes, though proteasome inhibitors completely arrest primary spermatocytes at metaphase I. Taken together, our results show that the canonical SAC signaling pathway is not required for second male meiotic division. We are currently investigating if additional mechanisms are required for monitoring chromosome segregation fidelity in second division.

P-181 Activity and functional conservation of neuropeptide signaling in C. elegans

Victoria Groß*[1], Miron Gershkovich*[2], Claudia Binder[1], Annette Beck-Sickinger[2], Torsten Schöneberg[1], Anette Kaiser*[2], Simone Prömel*[1]

[1]*Rudolf Schönheimer Institute of Biochemistry, Medical Faculty, Leipzig University, Leipzig, Germany*

[2]*Institute of Biochemistry, Faculty of Biosciences, Pharmacy and Psychology, Leipzig University, Leipzig, Germany*

Neuropeptide receptors (NPR) play essential roles in physiological processes such as anxiety or food intake. The neuropeptidergic system is conserved in vertebrates and invertebrates. NPRs belong to the family of G-protein coupled receptors (GPCRs) which transduce extracellular cues into cells. Their activity is regulated by a multitude of neuropeptides forming an elaborate temporally as well as spatially regulated network. In vitro studies have shown that different neuropeptides and variants of neuropeptide receptors switch between specific signal pathways. Due to this complexity and accompanying redundancies specific receptor-peptide interactions and their impact are challenging to study, especially in a vertebrate system. The nematode Caenorhabditis elegans is an excellent model for in vivo studies on the neuropeptidergic system and especially on the functional relevance of a specific NRP activation and molecular consequences. Our study aims at delineating the role of specific peptide-receptor interactions in various contexts, transferring information on signal transduction obtained from mammalian systems to C. elegans and investigate functional conservation. Signal capacities of C. elegans neuropeptides and receptors displaying a high sequence homology to members of the mammalian neuropeptide Y and RF-amide family were analysed in vitro and potential cross-activation of C. elegans receptors by mammalian neuropeptides and vice versa was examined. Our data suggest that several members of a set of human neuropeptide Y and RF-amide receptors (Y1, Y2, Y4, Y5, NPFF1R, NPFF2R, PrRPR, QRFPR) are not only activated by their endogenous peptides but also by the C. elegans peptides FLP-18 and FLP-21. Consistently, C. elegans neuropeptide receptors can be cross-activated. These findings demonstrate that both systems are highly conserved and can subsequently be utilised in the analysis of the effects of receptor-peptide interaction to bypass certain endogenous redundancies. Ultimately, our study will contribute to the understanding on how specific neuropeptide receptor signals are translated into physiological function.

P-185 Depletion of a neuronally expressed putative selenium binding protein ortholog induces stress resistance in C. elegans

Karl Köhnlein[1], Nadine Urban[1], David Guerrero-Gómez[2], Pavel Urbanek[1], Holger Steinbrenner[1], Christoph Kaether[3], Martin Srayko[4], Antonio Miranda-Vizuete[2], Lars-Oliver Klotz[1]

[1]*Institute of Nutrition, Department of Nutrigenomics, Friedrich-Schiller-Universität Jena, Germany*
[2]*Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Sevilla, Spain*
[3]*Leibniz Institute on Aging – Fritz Lipmann Institute, Jena, Germany*
[4]*University of Alberta, Faculty of Science, Edmonton, Canada*
Selenium binding proteins do not contain selenium in the form of selenocysteine or selenomethionine but as inorganic selenium bound to the protein. In mammals, the major protein of this kind is selenium binding protein-1 (SELENBP1). Its function is not well understood but it is thought to be involved in the regulation of cellular stress defense and is strongly downregulated in many tumor tissues. In C. elegans at least two ORFs encode putative selenium binding proteins, including R11G10.2, which encodes a protein that is 36% homologous to human SELENBP1 and also has a cysteine residue conserved that is believed to bind selenite in human SELENBP1. R11G10.2 is therefore referred to as ceSELENBP-1 here. We hypothesized that ceSELENBP-1 might be involved in the regulation of cellular defense mechanisms in the worm. Surprisingly, knock-down of ceselenbp-1 resulted in a significantly increased, rather than decreased, life span, improved locomotion and an increased resistance to the redox-cycler paraquat. Moreover, knock-down of ceselenbp-1 also resulted in a significant lifespan extension in daf-16- and skn-1 mutants, implying that these key players in C. elegans stress response are not involved in the observed lifespan modulation. CeSELENBP-1 is predominantly expressed in a subset of head neurons, which we will identify by comparison to known neuronal markers. Transcriptome analysis (RNAseq) of worms following ceselenbp-1 knock down suggests that ceSELENBP-1 is involved in the regulation of proteasomal composition, protein ubiquitination and the unfolded protein response (UPR). These findings are in line with literature data on the role of proteostasis in C. elegans lifespan regulation. The exact mechanism of ceSELENBP-1 action in this context remains to be elucidated. Supported by Deutsche Forschungsgemeinschaft (DFG), Bonn, Germany, through RTG 2155 (ProMoAge).

P-187 DAF-16 produces a fold-change, not an absolute effect, on C. elegans survival under aging and stress.

Nicholas Stroustrup
Centre for Genomic Regulation
Daf-16 is the only C. elegans FOXO forkhead transcription factor ortholog. Across eukaryotes, FOXO transcription factors promote organismal resistance to multiple environmental stresses, and are famous for their role in aging. FOXO transcription factors are central transcriptional mediators of the insulin/IGF signaling pathway and in C. elegans, daf-16 influences the expression level of about one fifth of the transcriptome (Murphy, 2003). It is not surprising, therefore, that daf-16 activity is under complex regulation—at the promoter and splicing level (Ruvkun 2001, Kwon 2010), as well as post-transcriptionally by ubiquitination, phosphorylation, methylation, and acetylation (Lin 2001, Li 2007, Fukamizu 2008, Chiang 2012). Daf-16 is also completely dispensable for C. elegans development and reproduction, allowing precise quantification of DAF-16's physiologic action by comparing daf-16 (+) animals to daf-16(mu86) null mutants. It is known that daf-16(mu86) mutants live short in many conditions, but recently, we found that daf-16(mu86) mutants in fact live a fixed proportion shorter than wildtype across a wide range of conditions (Stroustrup, 2016). Between 20 °C and 28 °C wild-type mean lifespan drops from twenty days to five days, and we find that daf-16(mu86) animals live a constant 25% ± 3 shorter across this range, independent of the absolute wildtype lifespan. Additionally, we find that exposure to concentrations of the oxidant tert-butyl hydroperoxide between 0.7mM to 10mM, drops wild-type mean lifespan from 20 days to eight hours, across which we find that daf-16(mu86) mutants live a fixed 20% ± 9 shorter than wildtype. These results suggest that the normal physiologic role of DAF-16 is to produce a fixed fold-change, not an absolute change, in the rate of physiologic processes that determine the timing of death. How this fold-change is accomplished at the mechanistic level remains unknown but has important implications on how we interpret the gene's physiologic action.

P-189 Temporal scaling during C. elegans post-embryonic development

Olga Filina, Rik Haagmans, Jeroen van Zon

AMOLF

It is essential that correct temporal order of cellular events is maintained during animal development. Interestingly, during post-embryonic development the rate of developmental progression can vary significantly with external conditions, such as food availability, diet and temperature. How timing of cellular events is impacted when the rate of development is changed is not known. We use a novel time-lapse microscopy approach to simultaneously measure the timing of oscillatory gene expression of molting cycle genes, seam cell division and cuticle shedding in individual C. elegans larvae, under environmental conditions and in mutants that change the timing of larval development. In particular, we examined lin-42/PERIOD mutants, which show strong variability in larval stage duration. We find that in all cases the timing of these developmental events scales with larval stage duration, i.e. their absolute timing is changed so that they occur at the same fraction of the larval stage, even if larval stage duration is strongly perturbed. Because of its homology to PERIOD, a key component of the circadian clock, lin-42 is assumed to play a key role in regulating timing of C. elegans development. However, our findings suggest that C. elegans possesses mechanisms that establish correct timing of events relative to the larval stage that are independent of LIN-42.

P-191 RNA Binding Proteins in stress resistance – a screen in C. elegans

Reza Esmaillie[1, 2], Tim Krüger[1, 2], Michael Ignarski[1, 2], Rene Neuhaus[1, 2], Francesca Fabretti[1, 2], Roman-Ulrich Müller[1, 2]

[1]*Department II of Internal Medicine and Center for Molecular Medicine Cologne (CMMC), University of Cologne*

[2]*Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), Nephrolab Cologne, University of Cologne*

RNA binding proteins (RBPs) play an important role in cell biology, regulating expression, stability and localization of all known RNA species. The importance of these proteins is underlined by the increasing body of evidence linking several hereditary diseases, developmental disorders and cancer with mutations in genes encoding RBPs. In the last decade, the list of known and putative RBPs has been increasing in size and complexity across species. Thanks to the development of techniques that allow crosslinking of RNA to interacting proteins followed by both RNA pulldown and mass spectrometry (RNA interactome capture), or immunoprecipitation and next generation sequencing (CLIP). Little is still known about the molecular function of many RBPs and their global dynamics in stress conditions. In this study we choose C. elegans as a model organism to address this complex biological question. Performing RNA interactome capture we identified 641 putative RBPs out of which 582 are conserved in mouse and human. We screened for RBPs involved in stress resistance pathways through heat stress resistance phenotyping using both mutants and RNA interference. In order to identify targets of the RBPs we established a modified CLIP protocol for C. elegans. We tagged RBPs endogenously using CRISPR/Cas9 technology and we performed immunoprecipitation and next generation sequencing. These results broaden the understanding of RBPs function during stress response in the nematode.

P-193 Characterization of the C.elegans ubiquitin-modified proteome (ubiquitinome)

Batool Ossareh-Nazari, Anthi Katsiarimpa, Luis Briseno-Roa, Jorge Merlet, Lionel Pintard

Cell Cycle and Development, Institut Jacques Monod, UMR7592 CNRS - Université Paris Diderot, Sorbonne Paris Cité, Paris, France.

Ubiquitination is a post-translational modification that typically signals protein degradation by the 26S proteasome, or modifies their function or localization. Alterations in the ubiquitin-proteolytic system have been implicated in a number of human diseases including cancers, cardiovascular diseases and neurodegenerative disorders. The ubiquitin modification system is highly conserved in C. elegans. Over the past two decades, a combination of forward genetics, reverse genetics, and genome-wide RNAi screens has provided information on the loss-of-function phenotypes for the majority of C. elegans ubiquitin pathway components and several substrates have been identified. However, in most cases, direct demonstration that a substrate is indeed

ubiquitinated and the identity of the ubiquitinated residues is lacking because it is extremely challenging to identify the exact modification sites under physiological conditions. To circumvent these problems we are implementing proteomic-based approach (ubiquitin-remnant profiling) to characterize the ubiquitin-modified proteome in C. elegans. We have so far identified around 8000 non-redundant ubiquitination sites in about 2000 non-redundant proteins. In parallel, we have generated a transgenic line expressing an RGS-6xHis-tagged version of ubiquitin, which allows purification of ubiquitinated proteins under denaturing conditions that preserve ubiquitination. We think that our approaches allowing identification of the protein ubiquitination sites will be a valuable resource for the C. elegans community.

P-195 Unbiased genetic screen to identify paternal factors involved in the regulation of the maternal mitochondrial transmission

Jihane Challita, Alice LeMorillon, Valeria Parrales, Aniela Zablocki, Jorge Merlet, Vincent Galy
Sorbonne Université, CNRS, Institut de Biologie Paris-Seine (IBPS), Developmental Biology Laboratory, UMR 7622, F-75005 Paris, France

The genomic DNA is inherited from both parents (one copy each) upon fertilization, making it a biparental transmission. Mitochondrial DNA, however, follows a uniparental transmission, typically maternal. It has been shown that the paternal mitochondria in C. elegans are degraded by postfertilization autophagy but the activating signal is yet to be determined (Al Rawi et al, 2011). In order to identify the paternal factors involved in the regulation of paternal/spermatic mitochondrial clearance, we will conduct an unbiased genetic screen on EMS mutagenized worms. We aim to identify male mutants able to transmit their mitochondria. To achieve this goal, we designed the screen on the basis of a positive selection of worms that keep the paternal mitochondrial genome. We use hermaphrodite worms that carry a genomic mutation leading to a dysfunctional respiratory chain and slow growth. These worms are crossed with mutated males harbouring a mitochondrial mutation that has the potential to rescue the slow growth phenotype. Furthermore, since this screen requires a large number of males, we also developed a high throughput method to rapidly and efficiently sort males from a large worm population. This screen will allow us to identify the marks that are specific to paternal mitochondria and could be recognized by a maternal mechanism that will activate the mechanism of autophagy.

P-197 The HIRA histone chaperone complex maintains normal cellular function in adult animals and protects against late-onset pleiotropic defects

Kirk Burkhart[1, 2], Anna Corrionero[1, 2], Steve Sando[1, 2], Bob Horvitz[1, 2]
[1]*HHMI*
[2]*Department of Biology, MIT*

Individual cells can function for remarkably long periods of time. For example, retrospective birth dating of human cells suggests that muscle cells can function for up to 15 years and that neurons can function for an entire lifetime. Aging and many aging-associated diseases are characterized by a progressive decline in cellular functions. The molecular mechanisms by which cells preserve their functions throughout an organism's lifespan are unclear. Here we report an essential role for HIRA-1 in maintaining normal cellular function in adult C. elegans. HIRA is an evolutionarily conserved histone chaperone that facilitates the deposition of the histone variant H3.3. We characterized mutants lacking hira-1 (the sole HIRA ortholog encoded in the C. elegans genome). Loss of hira-1 results in age-dependent pleiotropic defects: whereas hira-1(-) larvae are healthy, hira-1(-) adults have defects in body size, pigmentation, feeding, and defecation. HIRA-1 localizes to nuclei, is broadly expressed, and functions in multiple cell types to protect against these age-dependent pleiotropic defects. hira-1 mutants also display a progressive decay in intestinal nuclear architecture and stage-specific misregulation of gene expression. We designed a mutagenesis screen to identify factors that function similarly to hira-1. In addition to identifying alleles of hira-1, this screen identified pqn-80. PQN-80 is the C. elegans ortholog of a core member of the HIRA complex (UBN-1 in humans and HPC2 in yeast), strongly suggesting that PQN-80 is a core member of the C. elegans HIRA complex. We posit that the C. elegans HIRA complex maintains normal nuclear architecture and gene expression to preserve normal cellular function in adult animals.

P-199 The Slit/Robo pathway component eva-1 and RhoGAP-containing domain gene 2RSSE.1 are novel Wnt target genes required for termination of neuroblast migration in C. elegans

Lorenzo Rella, Euclides E. Fernandes Pòvoa, Annabel L.P. Ebbing, Marco C. Betist, Hendrik C. Korswagen
Hubrecht Institute

Cell migration is a fundamental process during development. Many studies have shed light on the mechanisms which regulate the movement of a cell, but molecular mechanisms that determines how cells terminate migration are still unclear. In order to investigate this specific cell behavior, we use the C. elegans QR neuroblast and descendants as a model to study termination of cell migration in vivo. The migration of these cells is regulated by non-canonical Wnt signaling, but the termination of their migration is triggered by a cell-intrinsic activation of the canonical/beta-catenin signaling pathway. In this work, we investigate about the downstream signaling mechanisms of canonical Wnt signaling in this process. Using a novel Q neuroblasts isolation technique, we were able to identify two novel targets of canonical Wnt signaling: the transmembrane protein EVA-1 and the RhoGAP-contaning protein 2RSSE.1. eva-1 acts in a slt-1-dependent manner in order to stop the migration of the QR.pa neuroblast; 2RSSE.1/RhoGAP, together with pix-1/RhoGEF, orchestrate termination of migration as effectors of a cross-talk mechanisms between canonical and non-canonical Wnt signaling pathways. From these results, we conclude that different mechanisms triggered by canonical Wnt signaling play a central role to ensure proper termination of cellular migration in vivo.

P-201 Deciphering the role of mitochondrial prohibitins as lifespan modulators of sgk-1mutants

Mercedes M. Pérez-Jiménez[1, 2], Blanca Hernando-Rodríguez[1, 2], Mª Jesús Rodríguez-Palero[1], Antoni Pla[1], Roxani Gatsi[1], Marta Artal-Sanz[1]
[1]*Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide, Sevilla, Spain*
[2]*These authors contributed equally to this work*

Prohibitins, PHB1 and PHB2, are two conserved proteins forming a ring-like macromolecular complex in the mitochondrial inner membrane. Their function is essential for embryo development, mitochondrial biogenesis and mitophagy. Depletion of PHB has pleiotropic phenotypes with a very peculiar effect on aging: lack of PHB decreases lifespan in a wild-type background, but increases the lifespan of different metabolically compromised long-lived mutants. The Serum and Glucocorticoid Kinase 1 (SGK-1) acts in different pathways, such us the insulin signalling and mTORC2 pathways. Depletion of prohibitins in sgk-1 mutants increases lifespan, which is inversely correlated with induction of the mitochondrial unfolded protein response (UPRmt): PHB depletion triggers a strong activation of the UPRmt in wild type worms, which is reduced by sgk-1 deletion. In this work, we analyse in detail the specific aspects required for the increased longevity of sgk-1(ok538) mutants upon prohibitin depletion. We show that sgk-1 deletion induces autophagy as well as the UPRmt. We present evidence that these two processes are essential for the increased lifespan of sgk-1(ok538) upon PHB depletion, while mitophagy does not play a role. Furthermore, we analyse the role of reactive oxygen species (ROS) and show that sgk-1 mutants present high levels of cytoplasmic ROS. Our results suggest that prohibitin depletion raises mitochondrial ROS levels. This effect in a specific preconditioned background with high cytoplasmic ROS, such as sgk-1 mutants, results in enhanced longevity.

P-203 RAB-6.2 regulates LET-23 EGFR-mediated vulval induction

Sarah Gagnon[1, 2], Kimberley Gauthier[1, 2], Christian Rocheleau[1, 2, 3]
[1]*Department of Anatomy and Cell Biology, McGill University, Canada*
[2]*Metabolic Disorders and Complications division, Centre for Translational Biology, Research Institute of the McGill University Health Centre, Canada*
[3]*Department of Medicine, McGill University, Canada*

The epidermal growth factor receptor (EGFR)/Ras/MAPK cascade promotes cell growth and proliferation. Overactivation of this pathway underlies many human cancers, and understanding its regulation is essential. Induction of the vulval cell fate in vulva precursor cells (VPC) of Caenorhabditis elegans relies on EGFR/Ras/MAPK

signaling. LET-23 EGFR basolateral localization, mediated by the LIN-2/LIN-7/LIN-10 protein complex, is required for receptor activation. Mutations in this complex cause loss of LET-23 basolateral localization and a vulvaless (Vul) phenotype. lin-10 overexpression (OE) rescues the Vul phenotype of lin-2 and lin-7 mutants, suggesting a function for LIN-10 outside of this complex. LIN-10 and the RAB-6.2 GTPase co-localize to the Golgi in C. elegans neurons and interact to regulate retrograde trafficking and recycling. We hypothesize that in the VPCs, RAB-6.2 positively regulates LET-23 signaling by recruiting LIN-10 to the trans-Golgi network, where it can promote LET-23 basolateral localization and activation. We tested RAB-6.2 function in LET-23 signaling by RNAi knockdown of pathway players in rab-6.2 animals. We also tested whether rab-6.2 knockout alters LET-23 and LIN-10 localization in VPCs using GFP-tagged proteins. Finally, we assessed whether rab-6.2 knockout suppresses lin-10(OE) rescue of lin-2 Vul animals. Although rab-6.2 worms exhibit wildtype induction, the Vul phenotype of lin-3 EGF knockdown is enhanced by rab-6.2 knockout, suggesting that RAB-6.2 positively regulates the pathway. While LET-23::GFP localization is not significantly altered VPCs of rab-6.2 worms, GFP::LIN-10 expression is lost, suggesting a role for RAB-6.2 in LIN-10 stability. Finally, rab-6.2 knockout alone rescues the lin-2 Vul phenotype, suggesting an antagonistic role for RAB-6.2 in LET-23 signaling. Our results suggest a role for RAB-6.2 in the regulation of LET-23 and LIN-10, however the mechanisms remain unclear. The conflicting evidence from lin-2 and lin-3(RNAi) experiments suggests that RAB-6.2 might function in the VPCs, and in the ligand-secreting cell to regulate vulval induction.

P-205 Stable maintenance of neuronal cell fate by the che-1 genetic switch

Joleen J.H. Traets, Jeroen S. van Zon

AMOLF, Science Park, Amsterdam, The Netherlands

It is an unsolved question how genetic switches that control cell fate remain in the correct state for the entire lifetime of an animal, despite stochastic variability on the molecular level. The transcription factor che-1 controls ASE neuron specification and cell fate maintenance in the nematode C. elegans. It induces hundreds of ASE-specific genes by binding ASE motifs within their promoter region. Crucially, che-1 also upregulates its own expression, thereby acting as a genetic switch. We used single molecule FISH to measure the expression level of che-1 as well as its target genes with single mRNA resolution. Surprisingly, we found that che-1 is expressed at low levels, ~5 mRNA/cell, at all stages of post-embryonic development. Interestingly, we found substantially higher levels, ~30 mRNA/cell, in the ASE neurons at its time of specification in the embryo, suggesting different strategies are used for induction versus maintenance of ASE fate. Since low mRNA levels are expected to give rise to significant fluctuations on the protein level, this raises the question how the che-1 switch avoids turning off spontaneously due to stochastic variability in CHE-1 protein level. To address this question, we use both smFISH and (time-lapse) measurements on fluorescently labelled CHE-1 to directly characterize its protein and mRNA life time as well as its absolute protein and mRNA levels. By combining these experimental results with stochastic simulations of the che-1 switch, we will identify the key strategies used by this switch to ensure maximal stability for the observed low expression levels.

P-207 Branched actin regulates Cadherin/HMR-1 trafficking

Sofya Borinskaya, Shashikala Sasidharan, Martha Soto

Department of Pathology and Laboratory Medicine, Rutgers – Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854.

Our lab has mainly focused on the dynamic movements of sheets of cells during embryonic morphogenesis. Genetic screens for morphogenesis mutants led us to focus on the WAVE family of branched actin regulators. We have shown, for example, that the sheet migration of epidermal ventral enclosure requires input from a pathway that includes Rac1/CED-10, the WAVE/SCAR/GEX complex and Arp2/3. Embryos and adults depleted of branched actin regulators, including the CED-10/WAVE/Arp2/3 module, show apical/basal defects that led us to test if branched actin regulates the assembly of a developing apical junction, and its maintenance. We found that during junction development, the apical accumulation of WAVE and Cadherin components is interdependent: Cadherin complex loss alters WAVE accumulation, and WAVE complex loss increases Cadherin accumulation. To determine

why Cadherin levels rise when WVE-1 is depleted, we used FRAP to analyze Cadherin dynamics and found that loss of WAVE as well as of the trafficking protein EHD-1/RME-1 increases Cadherin dynamics. EM studies in adults depleted of branched actin regulators support that WVE-1 maintains established junctions, possibly through its trafficking effect on Cadherin. By using Cadherin as a cargo to study trafficking in a developing epithelium, and in the maintenance of a mature epithelium, we are testing a model for junction regulation where branched actin regulators work with known endocytosis proteins to promote distinct steps of Cadherin transport.

P-209 RNA Polymerase II CTD serine 2 phosphorylation regulates developmental arrest in C. elegans

Fanelie Bauer[1], Clement Cassart[1], Carlo Yague-Sanz[1], Francesca Palladino[2], Valerie Robert[2], Damien Hermand[1]

[1]URPHYM-GEMO, The University of Namur, Belgium

[2]ENS Lyon, France

Many steps in gene expression are coordinated by the C-terminal domain (CTD) of the largest subunit of Pol II. Best characterized is the dual gradient of CTD serines 2 (S2P) and 5 (S5P) phosphorylation pattern during transcription, which is required for the association of RNA processing complexes. Whether the patterns that underlie the CTD phosphorylation code are universally required across all transcribed units is an outstanding question. Our previous work in yeast revealed that S2P is not required for vegetative growth but rather affects specific developmental processes. How the modifications of the CTD are integrated during development of multicellular organisms is an unexplored area. In order to address, this question, we have generated an analogue-sensitive (-as) version of the CTD S2 kinase Cdk12 expressed from the endogenous locus in C. elegans, which allows the deprivation of the bulk of CTD S2 phosphorylation in vivo within minutes. This revealed that embryogenesis can occur normally without detectable level of CTD S2P but the worms arrest their development at the L1 stage. The developmental arrest is fully reversible upon removal of the inhibitor. The worm postembryonic development is governed by nutrient availability and the inhibition of Cdk12 and S2P mimicks starvation. RNA-Seq revealed that only a subset of genes activated to exit the arrest required S2P. These genes are located in position 2 and over within operons and ChIP-Seq show that S2P is required to link the polyadenylation of the transcript in position 1, which is not affected, with the trans-splicing of the second transcript through the S2P-dependent recruitment of the CstF (Cleavage Stimulation Factor) complex. Our data clearly indicate that rather than affecting transcription globally, S2P is critical for the postembryonic L1 developmental transition. This suggests that there is a disconnect between the universal pattern of CTD S2P and its biological relevance.

P-211 Crosstalk between the ARP2/3 Complex and Formin Ensures Timely Cytokinesis

Fung-Yi Chan[1, 2], Ana M. Silva[1, 2], Joana Saramago[1, 2], Ana X. Carvalho[1, 2]

[1]i3S, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal.

[2]Instituto de Biologia Molecular e Celular – IBMC, Porto, Portugal.

Cytokinesis, the process that completes cell division by physically partitioning the mother cell into two daughter cells, requires precise spatio-temporal control of acto-myosin network-based contractility at the cell cortex. At the cell equator, non-branched actin filaments arrange in an anti-parallel manner to form a contractile ring that drives partitioning, while actin filaments outside the cell equator arrange in a crosslinked meshwork that is under tension and likely resists contractile ring closure. How these two actin network architectures are established and regulated in animals remains poorly understood. Here, we dissect how the formin CYK-1 and the ARP2/3 complex, nucleators of non-branched and branched actin filaments, respectively, contribute to actin filament network organization and function during cytokinesis in the C. elegans early embryo. Using quantitative assays based on live-imaging, we established that CYK-1 and ARP2/3 are the main actin filament nucleators during cytokinesis that generate distinct network architectures. We showed that the actions of the two nucleators are spatially segregated: CYK-1 is essential at the cell equator for structural integrity of the constricting contractile ring and for setting constriction speed, while the ARP2/3 complex determines the level of cortical tension and prevents abnormal accumulation of CYK-1 activity outside the contractile ring. Engineered ARP2/3 mutants that delay disassembly of the branched actin filament network specifically affect events at the cell equator, supporting the idea that ARP2/3 activity must be locally inhibited so as not to interfere with formin activity. Our results define

distinct roles for CYK-1 and the ARP2/3 complex during cytokinesis and uncover functional interplay between the two actin filament nucleators.

P-217 Characterization of the role(s) of the C. elegans Ceh-6 and Sox-2 transcription factors during a cellular transdifferentiation event.

Anne Daulny[1], Arnaud Ahier[2], David Rodriguez[1], Thomas Le Gal[1], Elena Morganti[3], Joshua Brickman[3, 4], Sophie Jarriault[1]

[1]Institut de Génétique et de Biologie Cellulaire et Moléculaire (IGBMC), Department of Development and Stem Cells CNRS UMR7104, INSERM U964, Université de Strasbourg, 1 rue Laurent Fries, 67404 Illkirch CU Strasbourg, France.

[2]Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, Brisbane, Australia

[3]The Novo Nordisk Foundation Center for Stem Cell Biology - DanStem, University of Copenhagen, 3B Blegdamsvej, DK-2200 Copenhagen N, Denmark.

[4]MRC Centre for Regenerative Medicine, Institute for Stem Cell Research, School of Biological Sciences, University of Edinburgh, 5 Little France Drive, Edinburgh EH16 4UU, UK

The Y cell of the C. elegans rectum has been shown to transdifferentiate from an epithelial to a neuronal cell identity during larval development. The initiation of this natural cell reprogramming event involves a step of dedifferentiation of the Y cell, where it loses its rectal identity, that requires the Sox-2 and Ceh-6 transcription factors (1). Sequence alignments show the strong conservation of the DNA-binding domains of Ceh-6 and Sox-2 with the ones of the mammalian Oct4 and Sox2 proteins respectively. We have found many shared properties between the C. elegans and the mammalian proteins: similarly to the direct interaction between Ceh-6 and Sox-2, Oct4 and Sox2 physically interacts (1). Oct4 and Ceh-6 form similar types of complexes called respectively NODE and NODE-like (1, 2). The Oct4/Sox2 and Ceh-6/Sox-2 heterodimers are both required for cells reprogramming events. Oct4 and Sox2 are master regulators in the generation of induced pluripotent stem cells (iPSCs), and are necessary for the maintenance of embryonic stem cells (ESCs) (3, 4). Despite all these similarities, important functional differences emerge between the Ceh-6/Sox-2 and Oct4/Sox2 heterodimers during the reprogramming events they govern. While Oct4/Sox2 is involved in driving dedifferentiation associated to pluripotency in iPSCs and ESCs, Ceh-6 and Sox-2 confer dedifferentiation capability to the Y cell but no widening of its cellular potential (1). We are investigating the role(s) of Ceh-6 and Sox-2 during the dedifferentiation step of the Y epithelial cell. In particular, we want to decipher the molecular mechanisms underlying the differences in functional abilities between Oct4/Sox2 and Ceh-6/Sox-2 during reprogramming. 1. Kagias K, et al. (2012) Proc Natl Acad Sci U S A 109(17):6596-6601. 2. Liang J, et al. (2008) Nature cell biology 10(6):731-739. 3. Boyer LA, et al. (2005) Cell 122(6):947-956. 4. Takahashi K & Yamanaka S (2006) Cell 126(4):663-676.

P-221 Unravelling Actin-Filament Crosslinkers diversity in vivo

Ana Filipa Sobral[1, 2], Fung Yi Chan[1, 2], Ana Xavier Carvalho[1, 2]

[1]i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal

[2]Instituto de Biologia Molecular e Celular – IBMC, Porto, Portugal

The actin cytoskeleton is essential for several vital cellular processes, including cell division, morphogenesis and motility/invasion. Actin filaments are interconnected by crosslinkers, which are main determinants of the architecture and dynamic properties of actin networks. How different actin filament crosslinkers contribute to a range of cellular processes in vivo is poorly explored. It is also unknown whether the expression of several crosslinkers reflects functional redundancy or a necessary diversification of the cells. We are dissecting the roles of 9 actin filament crosslinkers in cellular contexts that depend on actin network rearrangements in C. elegans, such as 1-cell embryos undergoing cytokinesis, oogenesis in the gonad, and muscle morphology. The ability of worms to produce viable and normal number of embryos as well as of moving are also being analyzed. Loss of function phenotypes are studied after RNAi-mediated depletion of each crosslinker or in mutant worms expressing “crosslinkers” without actin binding capacity. We find that perturbation of crosslinkers’ function in general affect worms’ brood size and can lead to embryonic lethality. Of those crosslinkers already tested, some

play a role during early embryonic cytokinesis, some are important for gonad actomyosin network organization and others are necessary for muscle organization and worm motility. So far, our results show that different crosslinkers play specific roles within the organism, which suggests that they contribute differently to the dynamic organization of actin architectures in vivo. We are currently testing whether crosslinkers can adjust their functionalities in situations where more than one crosslinker's function is affected.

P-223 Characterization of helminth complexes I and II of the ETC and analysis of their relevance in malate dismutation

Lucía Otero[1], Cecilia Martínez[1], Exequiel Barrera[2], Sergio Pantano[2], Gustavo Salinas[1, 3]

[1]*Worm Biology Laboratory, Institut Pasteur de Montevideo*

[2]*BioMolecular Simulation Laboratory, Institut Pasteur de Montevideo*

[3]*Faculty of Chemistry, Universidad de la Republica*

Helminths are subjected to environments where oxygen tension varies, to which they adapt using different energy harvesting pathways. Under hypoxia they primarily use the malate dismutation pathway. During malate dismutation, complex I (CI) of the electron transfer chain transfers (ETC) electrons to complex II (CII) through rhodoquinol. CII functions as fumarate reductase and not as succinate dehydrogenase as it does in aerobiosis. In the parasitic nematode Ascaris suum, CII subunit composition differs depending on oxygen tension. Nevertheless, CII subunit composition and the reactions catalyzed in normoxia and hypoxia are not fully understood in other helminths, and CI composition has not been studied in this context in helminths yet. We analyzed over 20 helminths genomes and transcriptomes, and found that there have been gene duplications and gene losses of some CII genes throughout the evolutionary history of nematodes and flatworms. We also found that some nematodes have duplicated one of the CI genes involved in the quinone-binding site. Therefore, there is no single evolutionary event associated with malate dismutation involving the ETC. C. elegans dismutates malate, thus serves as a model to understand this pathway. It has duplicated the gene that codifies for subunit A of CII and one of quinone-binding site of CI. By docking studies of C. elegans and A. suum CII we identified the residues involved in the interaction quinone-CII. These amino acids are conserved between the two organisms. When analyzing the exometabolome through 1H-NMR and HPLC we found significant differences between normoxic and anoxic conditions, like the presence of lactate, succinate, fumarate and propionate in anoxic but not in normoxic conditions. Assays with mutant strains will be performed to asses if any of these duplicated genes are essential for malate dismutation. Enzymatic assays are being optimized, and mitochondrial proteomic analysis in normoxia vs anoxia is in progress.

P-225 The Adhesion GPCR LAT-1 controls oocyte maturation and sperm guidance in C. elegans

Daniel Matúš, Franziska Fiedler, Julia Luterán, Claudia Binder, Torsten Schöneberg, Simone Prömel

Rudolf-Schönheimer-Institute for Biochemistry, Medical Faculty, University Leipzig Leipzig, Germany

Reproduction is one of the most essential mechanisms of organisms to preserve their species and is predominantly warranted by development of functional germ cells and fertilisation. The mechanisms controlling both processes are interlinked and highly complex. Recently, we identified the Adhesion GPCR LAT-1 to have a function in reproduction of C. elegans. A lat-1 null mutant displays a reduced number of laid eggs and an increase in unfertilised oocytes. Phenotypic analyses showed that these defects are caused in parts by a slight gonad hyperplasia accompanied by an elevated number of mitoses. Further, an increased level of apoptosis was observed which can be speculated to be a compensatory mechanism for the increased proliferation. Produced oocytes in lat-1-deficient nematodes are smaller and their quality is reduced compared to wild-type oocytes. Mating analyses of wild-type and lat-1 mutant individuals revealed that LAT-1 also regulates sperm guidance. Together, both defects account for the reduced fertility of lat-1 mutant nematodes. We show that the effects of LAT-1 on oocyte maturation and sperm guidance are cell non-autonomous as the receptor is not present in germ cells but in cells of the somatic gonad such as the distal tip cell and gonadal sheath cells. Consistent with this notion, expressing the receptor specifically in the distal tip cell of nematodes with a lat-1 null background rescues both observed defects. Interestingly, LAT-1 exerts its role from the distal tip cell not in a classical way via G protein

signals as only the extracellular N terminus of the receptor is sufficient for LAT-1 function, thus indicating a unique signalling mechanism. Taken together, our data show that the Adhesion GPCR LAT-1 is a novel player in fertility of C. elegans which is involved in oocyte development and sperm guidance via a non-GPCR mechanism.

P-227 Using designer receptors (DREADDs) to decipher G protein signalling pathways in C. elegans

Franziska Fiedler, Jana Winkler, Claudia Binder, Torsten Schöneberg, Doreen Thor, Simone Prömel
Rudolf Schönheimer Institute of Biochemistry, Medical Faculty, Leipzig University, Leipzig, Germany

G-protein signalling is an evolutionary conserved concept highlighting its fundamental impact on developmental and functional processes. To understand and control dynamics and kinetics of the mechanisms involved, pharmacological modulation of specific G protein pathways is advantageous but difficult due to a lack in accessibility and regulation. Recently, Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) have been developed to study G protein signalling pathways in vivo. DREADDs are based on muscarinic acetylcholine receptors and only activated by the inert drug clozapine-N-oxide. This concept enables a spatiotemporal control of GPCR pathways rendering powerful tools to study effects of G protein cascades. The usefulness of the DREADD technology has been demonstrated in mammalian systems, but due to their complexity investigation of mechanisms and their interplay sometimes proves impossible. To overcome these limitations, DREADDs for C. elegans would be favourable. Many G protein-signalling pathways are highly conserved making the nematode an ideal model to study biological consequences of G protein activation in vivo. In this study we used the homolog of the muscarinic acetylcholine receptor M3 in C. elegans (GAR-3) to develop the first C. elegans-specific DREADD. It triggers the Gq protein pathway and is activated by CNO comparable to established designer receptors. The functionality of this DREADD was shown in vivo modulating mating behaviour in a gar-3-deficient worm strain which displays several defects. These can be rescued by inserting and activating the newly designed DREADD and thus, complementing the signal normally conveyed by GAR-3, demonstrating the feasibility of this approach. These results establishing the functionality of a C. elegans-specific DREADD are a first step towards a set of DREADDs as tools to study questions regarding the impact of G protein signals and their interplay in vivo. They might lead the way to a general application of these receptors in C. elegans.

P-229 Developmental dynamics of gene expression and alternative polyadenylation in the Caenorhabditis elegans germline

Sean West[1], Desirea Mecenas[1], Michelle Gutwein[1], David Aristizabal-Corrales[1], Fabio Piano[1, 2], Kristin Gunsalus[1, 2]

[1]*Center for Genomics & Systems Biology, Department of Biology, New York University, New York, USA*
[2]*Center for Genomics & Systems Biology, NYU Abu Dhabi, Saadiyat Island, Abu Dhabi, United Arab Emirates.*

The 3' untranslated regions (UTRs) of mRNAs play a major role in post-transcriptional regulation of gene expression. Selection of transcript cleavage and polyadenylation sites is a dynamic process that produces multiple transcript isoforms for the same gene within and across different cell types. Using LITE-Seq, a new quantitative method to capture transcript 3' ends expressed in vivo, we have characterized sex- and cell type-specific transcriptome-wide changes in gene expression and 3' UTR diversity in Caenorhabditis elegans germline cells undergoing proliferation and differentiation. We show that nearly half of germline transcripts are alternatively polyadenylated, that differential regulation of endogenous 3' UTR variants is common, and that alternative isoforms direct distinct spatiotemporal protein expression patterns in vivo. Dynamic expression profiling also reveals temporal regulation of X-linked gene expression, selective stabilization of transcripts, and strong evidence for a novel developmental program that promotes nucleolar dissolution in oocytes. We show that the RNA-binding protein NCL-1/Brat is a posttranscriptional regulator of numerous ribosome-related transcripts that acts through specific U-rich binding motifs to down-regulate mRNAs encoding ribosomal protein subunits, rRNA processing factors, and tRNA synthetases. These results highlight the pervasive nature and functional potential of patterned gene and isoform expression during early animal development.

P-231 LIN-10 can promote LET-23 EGFR signalling independently of LIN-2 and LIN-7

Kimberley Gauthier[1, 2], Christian Rocheleau[1, 2, 3]

[1]*Department of Anatomy and Cell Biology, McGill University, Canada*

[2]*Metabolic Disorders and Complications division, Centre for Translational Biology, Research Institute of the McGill University Health Centre, Canada*

[3]*Department of Medicine, McGill University, Canada*

The spatial organization of signal transduction cascades is critical for regulating cell signalling and function. This is particularly evident in Caenorhabditis elegans vulva precursor cells (VPCs), where Epidermal Growth Factor Receptor (LET-23 EGFR) signalling from the basolateral membrane is required for vulva cell fate induction. The LIN-2 CASK/LIN-7 Veli/LIN-10 Mint complex mediates basolateral LET-23 localization through an unknown mechanism, and disruption of this complex results in exclusive apical localization of LET-23 and a vulvaless (Vul) phenotype. We previously characterized a pathway consisting of ARF GTPases and AP-1, a Golgi-associated clathrin adaptor complex, that antagonizes basolateral localization of LET-23 and negatively regulates signalling. Interestingly, mammalian Mint proteins (LIN-10) bind Arf GTPases at the trans-Golgi network and may serve as an adaptor protein analogous to the AP-1 complex. I hypothesize that the LIN-2/7/10 complex competes with the ARF/AP-1 ensemble at the Golgi or recycling endosomes for LET-23 binding to mediate basolateral transport of LET-23. I found that GFP::LIN-10 localizes to cytoplasmic foci in the VPCs, consistent with Golgi localization as seen in neurons. LIN-10 localization is independent of LIN-2, LIN-7, and ARF, and is mediated by the C-terminal domains of LIN-10 that also mediate Arf binding in Mint. Furthermore, LIN-10 colocalizes with ARF-1.2 in the VPCs. Unexpectedly, I found that LIN-10 overexpression rescues the lin-2 and lin-7 Vul phenotypes via its C-terminal domains, and partly restores basolateral LET-23 localization in a lin-2 mutant, indicating that LIN-10 can promote LET-23 signalling and trafficking independently of its complex. Moreover, LIN-10 overexpression disrupts the wild-type polarized localization of LET-23, similar to downregulation of the ARF/AP-1 pathway. Going forward, I will test if LIN-10 independently promotes LET-23 signalling by interacting with ARFs, possibly disrupting the ARF/AP-1 pathway. The results suggest a novel pathway whereby Golgi-associated proteins coordinate polarized targeting of LET-23 to regulate cellular signaling pathways.

P-233 An artificial eggshell for the study of mechanical functions of eggshell in C. elegans development

Akiko Hatakeyama[1], Asako Sato[2], Yo Tanaka[2], Shuichi Onami[1]

[1]*Laboratory for Developmental Dynamics, RIKEN Center for Biosystems Dynamics Research, Japan*

[2]*Laboratory for Integrated Biodevice, RIKEN Center for Biosystems Dynamics Research, Japan*

A nematode embryo is covered with a chitinous eggshell, which prevents polyspermy, regulates spatial arrangement of blastomeres, and provides physical protection to embryo until hatching. However, the role of the eggshell in development at later stages remains to be clarified. We found that L1 arrests hatched from eggs without eggshell but with permeability barrier was about 10% shorter in the body length than that from intact eggs in C. elegans. This result implies that eggshell plays an important role in the embryonic elongation. Mechanical support by eggshell to embryos may be involved in the body length regulation. To test this hypothesis, we developed a microdevice as artificial eggshell. The device consists of three parts: 1) A polydimethylsiloxane (PDMS) substrate with microwells, which are as small as normal embryos in size, 2) a PDMS substrate with a suction channel, and 3) a polycarbonate pore membrane sandwiched by the two substrates. Eggs can be captured into the microwells efficiently by gentle suction. We removed eggshell from eggs with leaving the permeability barrier, and then incubated them inside wells in the device. As a result, we found that the body length of L1 arrests hatched from the incubated eggs is comparable to that from intact eggs. This result supports our hypothesis of the mechanical function of eggshell.

P-235 Germline signals repress signalling and metabolic programmes in C. elegans

Abraham Mains, Janna Hastings, Boo Virk, Olivia Casanueva

Babraham Institute (Cambridge, UK)

The pronounced longevity of worms, which lack germline stem cells (GSCs) was first described by Hsin & Kenyon in 1999 using laser ablation of GSC precursor cells. They proposed a model whereby the GSCs antagonize a life-extending signal from the somatic gonad that is only apparent when the GSCs are eliminated but the somatic gonad remains intact. Subsequent studies identified a hormone signalling pathway mediated by the steroid hormone, Dafachronic Acid, which was not only essential for this so-called "gonadal longevity", but also played a significant role in regulating normal reproductive development and metabolism {Gerisch et al 2007, Berman & Kenyon 2006}. Worms lacking GSCs display an unusual concurrence of pro-longevity processes, including reduced insulin and TOR signalling, increased PHA-4/FOXA and SKN-1/Nrf signalling, mito-hormesis, and a host of metabolic changes including increased autophagy, lipolysis, and fatty acid desaturation. In order to investigate how the gonad might be coordinating these processes over time, we profiled the transcriptome at high temporal resolution over the pre-morbid lifespan of three strains of C. elegans. The three selected strains have different gonadal composition: glp-1(e2144), which lacks GSCs, gon-2(q388); gem-1(bc364), which develop a degenerate germline containing sperm and oocytes, and fem-3(q20), which produces only sperm. Our data shows that expression of germline genes is highly and significantly anti-correlated to genes enriched for terms relating to cell signalling and metabolism. Further exploration of our data also indicates that animals lacking GSCs display robust dramatic shifts in metabolic gene expression over day 1 to day 4 of adulthood indicating a substantial metabolic remodelling in response to GSC absence. In addition, we show for the first time that DAF-16 regulates competing programmes of mitosis and metabolism in response to signals from the germline.

P-237 Involvement of epigenetic factors and metabolism in pluripotency maintenance in C. elegans

Francesca Coraggio[1, 2], Ringo Püschel[1, 2], Alisha Marti[1], Peter Meister[1]

[1]*Institute of Cell Biology, University of Bern, Switzerland*

[2]*Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland*

A promising therapy for degenerative diseases is the replacement of the deficient cells by injection of in vitro differentiated progenitors. Differentiation is a crucial step since incompletely differentiated cells could lead to the formation of teratomas. Understanding which signalling pathways and epigenetic determinants maintain and drive cell fate is therefore of prime importance for regenerative medicine. Using muscle transdifferentiation ectopically induced by the expression of a single transcription factor, we test cell plasticity in fully differentiated L1 animals. We find that the silent histone mark H3K27 methylation deposited by the MES/Polycomb complex is essential to restrict plasticity in fully differentiated L1 animals. In the absence of this mark, animals completely arrest development upon fate challenge. Furthermore, muscle and neurons undergo division while seam cells divide without replicating their DNA, leading to catastrophic mitosis. As most animals arrest development, we used this system to screen for plasticity enhancers, which knock-down would suppress developmental arrest. We find that the Notch signalling pathway can rescue arrest upon fate challenge, suggesting Notch acts to enhance plasticity in vivo. Surprisingly, cell plasticity additionally depends on the metabolic state of the animal: while fed animals arrest development, starvation renders animals insensitive to fate challenges and rescues all phenotypes. In addition, mutations in daf-2, the receptor of the insulin/IGF pathway, renders animals sensitive to cell fate challenges in the absence of food. We are currently investigating how food and Notch signalling are connected, linking metabolism and cell fate plasticity.

P-239 A novel model to visualize and quantify amyloid-beta fibrilization in vivo

Christian Gallrein[1], Manuel Iburg[1], Chetan Poudel[2], Sara Wagner-Valladolid[2], Gabriele Kaminski Schierle[2], Janine Kirstein[1]

[1]*Leibniz-Research Institute for Molecular Pharmacology, Robert-Roessle-Str. 10 13125 Berlin, Germany*

[2]*Department of Chemical Engineering and Biotechnology, University of Cambridge, Philippa Fawcett Drive, Cambridge CB3 0AS, United Kingdom*

Alzheimer's Disease (AD) is one of the most commonly occurring age associated forms of dementia. It exhibits a characteristic accumulation and aggregation of the peptide and protein Amyloid-beta and hyperphosphorylated Tau. Such deposits induce proteotoxic - and oxidative stress. Perturbance of protein homeostasis occurs in the

presence of amyloids and metastable proteins can precipitate or co-aggregate with amyloids leading to depletion of essential cellular proteins and therefore result in cytotoxicity. Conventional models are based on fluorescent fusion proteins such as GFP with the respective amyloid protein. However, this will likely affect the conformation, interaction with potential modifiers and susceptibility to remodelling and turnover. Omitting a fluorescent tag would on the other hand prevent a visualization of the aggregation propensity. To overcome this obstacle I designed a single operon to express Amyloid-beta and mScarlet-Amyloid-beta. The mScarlet-Amyloid-beta will incorporate into the amyloids in a sub-stoichiometric manner and thereby label the fibrils without perturbing the native amyloid beta-sheet structure or the kinetics of the A fibril formation. This is the first model to visualize Amyloid-beta fibrilization in neurons of a living organism in a non-invasive manner with the progression of ageing. Using this model I could observe the expected accumulation of fluorescent proteins into foci that can be stained with Thioflavin T. To quantify the fibrilization in situ and without any further staining I employed fluorescence lifetime microscopy (FLIM). Fluorophores are quenched, when they are in close vicinity of amyloid structures, which will result in a decrease of fluorescence lifetime. I could observe an inverse correlation between ageing and the fluorescence lifetime of A aggregates. The effect of modifiers of Amyloid-beta fibrilization such as molecular chaperones are currently being analysed. Together, sub-stoichiometric tagging and FLIM offer the possibility to study amyloid fibrilization in a non-invasive manner in vivo.

P-241 Dissecting the mechanism of transcriptional priming, a strategy for cellular diversification

Julien Charest, Thomas Daniele, Jingkui Wang, Luisa Cochella

Research Institute of Molecular Pathology (IMP), Vienna, Austria

Development occurs through a succession of gene-regulatory events that ultimately define the identity of an animal different cell types. Early regulatory events, such as the expression of a transcription factor (TF), can be transient. Yet, in a few reported cases, transient transcriptional activation of a locus has a lasting impact on its competence for further activation by a second TF, a phenomenon termed transcriptional priming. This two-step transcriptional strategy is not only important for robust gene activation, but also for diversification of transcriptional programs. Because activation of such a locus requires two temporally separated inputs, a cell population expressing a shared TF will differently activate that gene depending on whether it was primed or not. The best-understood example of this strategy being used for cellular diversification is provided by the ASE neuron pair. The ASE Left and Right neurons share a number of properties, provided by symmetric expression of a terminal selector TF, CHE-1. However, these neurons are functionally asymmetric due to expression of a miRNA, lsy-6, in ASEL but not ASER. While CHE-1 is necessary for lsy-6 expression in ASEL, it is unable to activate lsy-6 in ASER. This is because lsy-6 is only primed in the blastomere giving rise to ASEL, by transient action of two TFs, TBX-37/38. Expression of lsy-6 in ASEL triggers an asymmetric regulatory program that determines molecular and functional asymmetries between the two neurons. The molecular mechanism by which “memory” of early TBX-37/38 activity results in a lsy-6 locus that is competent for robust transcription, 5 cell divisions later, is still unclear. Here, we test a number of hypotheses using a combination of ChIP-seq, RNA-seq, lineaging of fluorescent reporters and degron-mediated manipulation. Our work will uncover the molecular basis for transcriptional priming, as well as potential additional lateral asymmetries in the worm’s nervous system.

P-243 The scent of a smoking gun: characterizing a new oomycete pathogen of C. elegans

Michael Fasseas, Clara Essmann, Michalis Barkoulas

Department of Life Sciences, Imperial College, London, United Kingdom

C. elegans has been shown to exhibit immune responses to a wide range of pathogens, such as viruses, bacteria and fungi. However, how C. elegans recognises the pathogens it encounters in order to mount suitable responses remains largely unclear. Recently, we expanded the list of known natural pathogens of C. elegans by introducing a new system in which an oomycete, Myzocytiopsis humicola, is able to induce an oomycete-specific immune response in C. elegans. This response is intriguing because it does not require infection and involves upregulation of chitinase-like (chil) family members, which we previously showed may act to modify the biochemical properties of the nematode cuticle, reducing the occurrence of pathogen attachment and thus antagonizing

infection. Here, we describe a second oomycete infecting C. elegans that belongs to the Haptoglossa genus. Unlike M. humicola, Haptoglossa sp. infects nematodes by firing a needle-like structure from a gun-cell inside the animal body through the cuticle. We present the pathogen life cycle from nematode infection to the release of new gun-cells. Using electron microscopy, we show that this new oomycete digests C. elegans from the inside in order to grow inside the host body cavity without disrupting at all the host cuticle. RNA-seq experiments suggest that infection by Haptoglossa sp. causes a distinct transcriptional response in C. elegans to that observed against other pathogens. However, this response includes the upregulation of chil gene family members and can occur by supplying a pathogen extract in the absence of infection, in a similar manner to M. humicola. Finally, we present our efforts towards producing a pathogen genome assembly. We anticipate that this new pathosystem will provide insights into the diversity of pathogen virulence factors, pathogen associated molecular patterns and host immune responses to oomycetes.

P-245 Impairment of the DLK-1 MAP Kinase Pathway Suppresses p25α Induced Neurodegeneration of Dopaminergic Neurons in C. elegans.

Anders Olsen[1], Marie Fuglsang[3], Katrine Stenz[3], Freja Sørensen[3], Katrine Vogt[1], Lotte Vestergaard[3], Maria Doitsidou[5], Frederik Vilhardt[4], Poul Henning Jensen[2]

[1]*Aalborg University Department of Chemistry and Bioscience Fredrik Bajers Vej 7H 9220 Aalborg*

[2]*Aarhus University, Dandrite and Department of Biomedicine*

[3]*Aarhus University, Department of molecular biology and genetics*

[4]*University of Copenhagen, DCMM*

[5]*The University of Edinburgh, Centre for Integrative Physiology*

Deposition of Lewy bodies in the dopaminergic neurons is a major pathological hallmark of Parkinson's disease. Lewy bodies contain a wide range of aggregated proteins among these are α-synuclein and Tubulin Polymerization Promoting Protein(TPPP/p25α). p25α promotes polymerization of tubulin into microtubules and can also induce aggregation of α-synuclein. We find that transgenic overexpression of human p25α in the dopaminergic neurons of C. elegans leads to dose- and age-dependent neurodegeneration. In order to uncover the underlying molecular mechanisms we performed a forward genetic suppressor screen. Following NGS sequencing and mapping using the CloudMap Galaxy based pipeline, dlk-1 and pmk-3 (p38) were found to be suppressors of p25α induced neurodegeneration. dlk-1 and pmk-3 are both part of the DLK-1 MAPK pathway which is essential for axonal regeneration. We also found that expression of p25α in PC12 cells caused a significant activation of p38. Furthermore, inhibition of p38 suppressed p25α mediated secretion of α-synuclein in PC12 cells. Therefore, the interaction between p25α and the DLK-1 pathway appears to be evolutionarily conserved. We propose that overexpression of p25α leads to altered microtubule dynamics which subsequently activates the DLK-1 MAPK pathway.

P-247 Dissecting dynactin's role in bipolar spindle assembly in somatic cells of the C. elegans early embryo

Tania M. Silva[1, 2], Patrícia A. Simões[1, 2], Reto Gassmann[1, 2]

[1]*Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto, Porto, Portugal*

[2]*I3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal*

The multi-subunit dynactin complex is a ubiquitous co-factor of the microtubule-based motor cytoplasmic dynein 1 (dynein), which has several important roles during cell division. In C. elegans, dynactin is essential for dynein-dependent assembly and positioning of the female meiotic spindle, as well as for the separation of centrosome attached to the male pronucleus and pronuclear migration in the zygote. Here, we use a degron-based strategy to investigate dynactin's contribution to spindle assembly in embryonic somatic cells, without interfering with earlier zygotic functions of dynactin. Transgene-encoded dynactin p150 (DNC-1) fused to the PIE-1 degron sequence is stably expressed in germ-line cells (including the zygote) and specifically degraded in somatic cells of the early embryo. RNAi-mediated replacement of endogenous DNC-1 with the DNC-1-degron fusion completely blocks bipolar spindle assembly in somatic cells of the 8-cell embryo, causing a substantial mitotic delay. Live-

imaging in 8-cell embryos reveals that DNC-1 is essential for centrosome separation in prophase, which depends to a large degree on pulling forces generated on astral microtubules by cortex-localized dynein-dynactin. A less penetrant RNAi regime for DNC-1 still impairs centrosome separation in prophase, but residual dynactin activity can drive subsequent incorporation of chromosomes into a bipolar spindle during prometaphase. Further experiments suggest that when centrosome do not fully separate during prophase, the kinetochore pool of dynein-dynactin becomes essential for bipolar spindle assembly during prometaphase. Our results thus reveal that dynactin assists dynein in bipolar spindle assembly during prometaphase, and that this function of dynactin can be uncoupled from its role in centrosome separation during prophase.

P-249 LIN-15B is necessary for enrichment of H3K9me2 on promoters of a subset of germline expressed genes in somatic cells

Andreas Rechtsteiner[2], Meghan Fealey[1], Thea Egelhofer[2], Susan Strome[2], Lisa Petrella[1]

[1]Dept. Biol Sci, Marquette University, Milwaukee, WI, USA

[2]Dept. MCD Biology, UC Santa Cruz, Santa Cruz, CA, USA

A central question in development is how gene expression programs are set up for proper cell fate acquisition. One challenge for this process is the repression of germline genes in all somatic tissues. Germline genes are not clustered in the genome and are biased to the center of chromosomes away from areas of heterochromatin and large areas of H3K27me3 modification. Therefore, repression of germline genes in somatic tissues cannot take advantage of these long range repressive chromatin environments. To study how germline genes are repressed in somatic tissues we looked at changes in histone modifications in three synMuv B mutants, lin-15B, lin-35, and lin-37, which all demonstrate ectopic expression of germline genes in somatic cells. We found that H3K9me2 was enriched on the promoters of germline genes, genes up-regulated in synMuv B mutants, and genes bound by synMuv B proteins in wild type L1s. Localization of H3K9me2 to gene promoters was largely lost in lin-15B mutants and to a lesser extent in lin-35 and lin-37 mutants. Genes that lost H3K9me2 promoter enrichment were correlated with germline expressed genes and genes up-regulated in synMuv B mutants. More broad H3K9me2 enrichment found on autosomal arms and across gene bodies was not disrupted in mutants. Interestingly, many genes that lost H3K9me2 promoter enrichment in mutants had both a H3K4me3 and H3K9me2 promoter peak in wild type animals and exclusively lost the H3K9me2 peak in mutants. Although our experiments cannot specifically address if these marks are present as a bivalent modification on the same promoter, promoters that can have both a H3K4me3 and H3K9me2 peak appear to be particularly labile for changes in expression during development. The enrichment of H3K9me2 to promoters of genes found in highly euchromatic areas of the genome may allow for gene specific reduction in expression in many tissues.

P-253 In search of higher-order genetic interactions in Caenorhabditis elegans

Katarzyna Toch, Marta Labocha

Institute of Environmental Sciences, Jagiellonan University, Kraków, Poland

Epistasis is a phenomenon in which the effect of one locus is not independent from the effect of another locus (or loci). It is predicted to play an important role in many evolutionary processes. This genetic interactions, even though still understudied, are well known for a long time now. Negligence of epistasis was probably due to the fact that additivity of alleles effects explains most of the variation in many traits. Notwithstanding, in recent high-throughput studies encompassing large portions of genomes, gene-gene interactions are observed in 5-7% of all gene pairs. Most studies explore epistasis as interactions between two genes and only few focus on higher-order genetic interactions (HGIs). HGIs are definitely more difficult to examine on a bigger scale, although latest studies show that HGIs explain from 6 to 32% of variation in fitness. What is more, it is claimed that only when higher-order epistasis is taken into account it is possible to accurately predict evolutionary trajectories. In my project, I plan to look for HGIs in Caenorhabditis elegans, using different wild type isolates. By applying 30 sets of two mutations, which are already known to interact epistatically, I am screening for differences among various genetic backgrounds, which serve me as a source of genetic diversity. My results will answer whether HGIs are frequent in C.elegans and address important questions about how much phenotypes depend on: i) single

mutations, ii) gene-gene interactions, and iii) genetic background. Those questions are still insufficiently addressed despite their importance not only in the field of biology but as well in medicine.

P-255 4D lineage screen for regulators of neuroblast identity: cell size and proneural gene expression

Thomas Mullan[1], Terry Felton[1], Nadin Memar[2], Osama Kasem[1], Justina Yueng[1], Ralf Schnabel[3], Richard Poole[1]

[1]*Department of Cell and Developmental Biology, University College London, London, United Kingdom*

[2]*Department Biologie II, Biozentrum der LMU München, Munich, Germany*

[3]*Institut fuer Genetik, TU Braunschweig, Braunschweig, Germany*

Neurogenesis necessitates the segregation of neurogenic potential within cell lineages and correct regulation of proneural gene expression. The Caenorhabditis elegans C lineage provides a model in which to study the molecular and cellular mechanisms regulating these processes as the otherwise bilaterally symmetric lineage gives rise to just two glutamatergic neurons on its left side, DVC and PVR. This is dependent on the expression of the proneural bHLH transcription factor hlh-14/ASCL1. Laser ablation of early blastomeres and lineage analysis of mex-3/MEX-3 mutants, in which AB derived lineages become C like, demonstrate that this is a lineage intrinsic mechanism. Taking advantage of the invariant cell lineage and single-cell resolution of C. elegans we are combining classical and modern techniques to perform a 4D-lineage screen of over 200 temperature sensitive embryonic lethal mutants for regulators of hlh-14/ASCL1. Identified from this screen, mutants of the Mediator complex component let-19/mdt-13 demonstrate loss of hlh-14 expression and precocious neuroblast division concomitant with volumetric symmetrisation of the division producing the C lineage neuroblasts. Measurement of C lineage divisions in a number of mutants highlights this asymmetric division; representing the point at which neural and non-neural ectodermal potential are segregated. The apoptotic neuroblast division polarity regulators pig-1/MELK and ham-1/STOX also display precocious neuroblast division with volumetric symmetrisation of this division, representing the first example of effects on division plane in divisions preceding the neuroblast divisions for these molecules. In asymmetric cell divisions, polarisation of the mother cell leads to asymmetric segregation of key cell fate regulators and production of two differently fated daughters. However, it is unclear if size alone has an influence on daughter cell fates. To investigate the specific role of cell size in our asymmetric division we are using a temperature sensitive allele of lin-5/NuMA to alter cleavage plane position without affecting polarity.

P-257 Exploring the Role of the Endoplasmic Reticulum Unfolded Protein Response (UPR-ER) in C. elegans Neurons

Nesem Ozbey[1], Christel Krueger[2], Soudabeh Imanikia[1], Ming Sheng[1], Olivia Casanueva[2], Rebecca Taylor[1]

[1]*MRC Laboratory of Molecular Biology, Cambridge, CB2 0QH, UK.*

[2]*Epigenetics Programme, The Babraham Institute, Cambridge, CB22 3AT, UK.*

The nervous system of *C. elegans* plays a role in the orchestration of systemic stress responses. One of these stress responses, the unfolded protein response of the endoplasmic reticulum (UPR-ER), is activated to re-establish proteostasis upon the detection of ER stress; overexpression of active, spliced XBP-1 (XBP-1s), a transcription factor that acts downstream of the UPR-ER kinase/endoribonuclease IRE-1, in the nervous system of *C. elegans* increases the lifespan and healthspan of worms through UPR-ER induction in the intestine. To investigate XBP-1s-dependent changes in the nervous system of these animals, we conducted tissue-specific RNASeq in neurons. This approach allowed us to characterise differentially regulated neuronal and synaptic components, which may mediate changes to the nervous system that cause the release of inter-tissue UPR-ER-activating signals. We also employed a candidate approach based on our previous finding that neurotransmitter secretion is required for cell non-autonomous UPR-ER activation, and identified positive & negative regulators of intestinal UPR-ER activation. We find that the specific neuronal circuitry required to activate the UPR-ER within the intestine can also generate behavioural phenotypes following neuronal XBP-1s overexpression. This suggests that inter-tissue UPR-ER activation, increased longevity and healthspan can be coordinately regulated with stress-responsive behaviour by the activation of this transcription factor in the nervous system. We are now further investigating this circuitry

to better understand the mechanistic links between the regulation of behaviour and longevity by neuronal XBP-1s activation.

P-259 Using C. elegans as a model for studying telomere maintenance pathways

Karim Hussain, Helder Ferreira
University of St Andrews, School of Biology, St Andrews, Scotland UK

Human ATRX is a Snf2 family chromatin remodelling enzyme and mutations of the ATRX gene are the underlying cause of ATRX syndrome, causing intellectual disability in male sufferers. The ATRX protein has also been shown to act as a tumour suppressor in a subset of particularly aggressive cancers that utilise an alternative, telomerase-independent pathway for telomere maintenance. While clearly important, loss of ATRX function alone is not sufficient for activation of this alternative lengthening of telomeres (ALT) pathway in cell line models. XNP-1 is the Caenorhabditis elegans homolog of ATRX and xnp-1(tm678) animals present a number of ALT-like phenotypes such as elevated levels of extra-chromosomal DNA (C-circles) and elongated telomeres compared to wildtype. Given that both C-circles and telomere length are hallmarks usually associated with ALT-positive cancers, xnp-1(tm678) constitutes a unique model that enables us to directly investigate the role XNP-1/ATRX plays in regulation of the ALT pathway. Here we characterise the ALT-like phenotypes associated with xnp-1(tm678) and apply forward genetics to identify potential genetic interactions of xnp-1.

P-261 An actin-dependent spindle positioning mechanism in the zygote of *Pristionchus pacificus*

Satoshi Namai, Daichi Sasaki, Asako Sugimoto
Graduate School of Life Sciences, Tohoku University

Developmental program is highly reproducible within species, but can be diverse even among relatively closely related species. Here, we compared the first cell division of *Pristionchus pacificus* with *Caenorhabditis elegans*. Although both zygotes undergo asymmetric cell division, we found that their cellular events, including cortical ruffling, pronuclear and mitotic spindle movements were strikingly different from each other. Immunofluorescence and live-imaging analysis of fluorescently labelled proteins revealed distinct spatial and temporal behaviors of microtubule and actin cytoskeletons in these two species. The mitotic spindle in the *C. elegans* zygote is formed in the center of embryos, and at anaphase it is displaced posteriorly with oscillation due to the asymmetric pulling forces on the posterior cell cortex. On the other hand, the spindle in *P. pacificus* zygotes were formed posteriorly and moved toward the center, then again rapidly displaced toward an "actin patch" transiently formed at the posterior cortex. This actin patch is disassembled shortly after its contact with the spindle pole. Comparison of the genome sequences revealed that *par-2* and a subset of essential genes essential for spindle positioning in *C. elegans* were absent in *P. pacificus*. Thus, distinct molecular mechanisms regulate polarity establishment and spindle positioning in *P. pacificus* and *C. elegans* embryos.

P-263 Understanding a mechanism of incomplete penetrance of human tumor suppressor gene PTEN by adaptive evolution of c. elegance

Anna Mellul, Irene Guberman, Idit Bloch, Yuval Tabach
Department of Developmental Biology and Cancer Research, The Institute For Medical Research-Israel-Canada, The Hebrew University-Hadassah Medical School, Jerusalem, Israel

Phosphatase and tensin homolog (PTEN) is a tumor suppressor gene that's frequently deleted or mutated in several human cancers, with medium to high penetrance. In this work, we focused on the Caenorhabditis elegans ortholog of the human PTEN, daf-18. Previous studies showed that human PTEN can functionally replace DAF-18 in C. elegans, suggesting that human PTEN and DAF-18 are functionally similar and that the regulation of PTEN is highly conserved in C. elegans. This makes C.elegans an excellent choice as a model organism, as it has both cellular complexity and conservation of disease pathways while being much simpler than other animal models. Despite many types of diseases manifesting incomplete penetrance, its mechanism isn't clear. Our hypothesis is that employment of adaptive or enforced evolution will allow us to find an alternative pathway or point mutation

which will allow c.elegans to overcome the depletion of daf-18. According to previous studies; there is about 70% penetrance after starvation of 72 hours. The worms were starved in every generation. We've started to see a change in penetrance after only two generations, and at about generation 45, there was a decrease of 50% in penetrance. We plan to keep the evolutionary process until the penetrance will decrease to the wt phenotype of about 5 %. Since the change was very fast we hypothesize some epigenetic modifications involved. To further study the mechanism, we plan to sequence both DNA and RNA from the different generations, and also perform an EMS screen as an enforced version of evolution. The understanding of incomplete penetrance of PTEN, and later other oncogenes, will enable to distinguish between mutation carriers. It will be possible to eliminate unnecessary suffering from those who are not at risk and to pay more attention to those who are at high risk.

P-265 Finding the Evolutionary Controls of Satiety Quiescence Behaviour using CeNDR

Bertalan Gyenes[1, 2, 3], André Brown[1, 2]

[1]*MRC London Institute of Medical Sciences*

[2]*Institute of Clinical Sciences, Faculty of Medicine, Imperial College London*

[3]*Department of Mathematics, Imperial College London*

Natural selection favours organisms that are flexible in the face of changing environmental conditions. As animal development and growth normally cannot be changed, flexibility is achieved by modifying behaviour. As feeding related genes often control multiple behaviours in a functionally coherent way in response to environmental challenges, they are interesting candidates for evolutionary control knobs. While classical genetics can identify genes, it cannot find the alleles relevant for evolution in the wild. To find out more about the genetic knobs tuning behaviour, we recorded more than 5000 individual worms from almost 200 strains from Caenorhabditis elegans Natural Diversity Resource (CeNDR), a collection of wild type isolates that includes the full genome sequence of each strain in addition to other information. Following starvation, worms that are refed become quiescent. This satiety quiescence behaviour is known to have significant genetic overlap with the mammalian postprandial somnolence behaviour, or 'food coma', so we expect some of the genes to be conserved and potentially provide insight into the human behaviour, as well. As the probability of satiety quiescence is a coarse measure of a potentially complex phenotype, we used high resolution tracking and multidimensional phenotyping to break the trait down into its parts. These phenotypic components are then individually associated to genetic variants, revealing new loci related to satiety quiescence and modularity in the phenotype-genotype map for this behaviour.

P-267 Characterization of a temperature-sensitive allele of egg-3

Amber Krauchunas, Kendall Flanagan, Peter Schweinsberg, Barth Grant, Andrew Singson

Rutgers University

Egg activation is the series of cellular events that transition a fertilized egg into a developing totipotent embryo. Egg activation events include the resumption and completion of meiosis, changes to the egg's outer coverings, and rearrangement of the actin cytoskeleton. It has been shown previously that egg-3 is a key regulator of egg activation in C. elegans. In the absence of EGG-3, fertilized eggs fail to form polar bodies, have defective F-actin dispersal, and lack a chitin egg shell (Maruyama et al., 2007). In addition, EGG-3 is necessary for proper localization of the other egg activation regulators EGG-4, EGG-5, CHS-1, and MBK-2 (Maruyama et al., 2007; Stitzel et al., 2007). We have carried out a forward genetic screen for new mutants with defects in sperm activation, fertilization, and/or egg activation. One of the mutants discovered in this screen is a novel temperature-sensitive allele of egg-3. The egg-3(as40) mutation is a single missense mutation that causes hermaphrodites to be marginally fertile at 16oC and sterile at 25oC. We find that stability and localization of this mutant form of EGG-3 is normal at both the permissive and non-permissive temperatures. Additional results will describe whether egg-3(as40) affects the localization of EGG-4, EGG-5, CHS-1, and MBK-2. We will also present the results of a preliminary screen to identify suppressors of egg-3(as40).

P-269 The effect of genetic background on seam cell development

Sneha Latha Koneru, Michalis Barkoulas

Imperial College London

Developmental studies in Caenorhabditis elegans have mostly been conducted using a single natural isolate, the lab reference strain N2. Therefore, it remains largely unclear whether our understanding of C. elegans biology based on N2 is truly representative at the species level. This also applies to the development of the seam cells, which are lateral epidermal cells that display stem cell-like properties because they undergo a series of asymmetric cell divisions to produce hypodermal and neural cells during larval development. To start addressing whether the genetic background may modify seam cell development, we introgressed a seam cell marker into divergent C. elegans isolates. We found that seam cell number is robust to standing genetic variation present in these natural isolates. However, we found that differences in seam cell number can be uncovered if the backgrounds of natural isolates are sensitized by introducing a genetic mutation. In particular, we present here that the expressivity of mutations in the GATA transcription factor egl-18, which is a target of the Wnt pathway, is higher in N2 than the CB4856 isolate. To map the underlying basis of this difference in expressivity, we made recombinant inbred lines between N2 and CB4856 carrying the sensitising mutation. Using bulk segregant analysis with next generation sequencing, we were able to identify multiple QTLs associated with the difference in seam cell number between the two isolates. We are currently building near isogenic lines to study how these QTLs affect seam cell number both individually and in combination, and discover the precise genetic changes influencing mutation expressivity.

P-271 Tissue-specific ChIP-seq to study genomic distribution of Histone variants and Trasncription Factors

I. Selman Bulut, Baris Tursun

Max Delbrück Center

Revealing the genomic distribution of transcription factors (TFs) and components of chromatin such as specific types of Histones known as Histone variants are important to understand biological processes including the control of spatial and temporal gene expression, fate acquisition and tissue development. However, chromatin factors such as the Histone H2A variant HTZ-1 (H2A.Z in mammals) and many TFs are not expressed in a cell-specific manner but, rather, more broadly. Analyzing the tissue-specific occupancy of these factors is of particular interest and may enable one to dissect regulatory programs driving cell type-specific processes such as differentiation but also TF-induced reprogramming of specific cell identities. Tissue-specific ChIP experiments are commonly achieved by cell dissociation and sorting by FACS, tissue dissection, or cell culture, which are, however, not well established in C. elegans due to the stable cuticle surrounding worms or difficulties in culturing postembryonic cells. We are applying a biotinylation-based ChIP-seq in C. elegans which is based on tissue-specific biotinylation of the target protein carrying the AVI tag. The AVI tag is recognized by the bacterial biotin ligase (BirA), which when expressed under a tissue-specific promoter, biotinylates the AVI tag only in the corresponding tissues or cell types. We tagged HTZ-1 with AVI, and use different strains where BirA is solely expressed in the epidermis, muscle, neurons, and germ cells..Using these strains, we performed serial ChIP-seq experiments to uncover HTZ-1 occupancy in in a tissue-specific manner. By analyzing these data we may tease apart the cell- and tissue-specific mechanisms of gene regulation by HTZ-1 and also TFs that we used in overexpression experiments.

P-273 Are mitochondrial DNA deletion or protein aggregation determinants of lifespan in Caenorhabditis elegans?

Zhuangli Yee[1], Lakshmi Narayanan Lakshmanan[2, 3], Rudiyanto Gunawan[2, 3], Barry Halliwell[1], Jan Gruber[1, 4]

[1]*Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore*

[2]*Institute for Chemical and Bioengineering, ETH Zurich, Zurich 8093, Switzerland*

[3]*Swiss Institute of Bioinformatics, Quartier Sorge – Batiment Genopode, 1015 Lausanne, Switzerland*

[4]*Ageing Research Laboratory, Science Division, Yale-NUS College, Singapore*

With age, nematodes experience functional decline like other organisms. Therefore, measurement of damage biomarkers in C. elegans may become useful in understanding the mitochondrial DNA (mtDNA) and protein damage mechanisms in ageing. The accumulation of damage, over a lifespan, can lead to deletions in the mitochondrial genome and also the aggregation of damaged proteins. However, it is unclear whether both mtDNA deletion and protein aggregation play a causative role in ageing. We developed and validated a real-time polymerase-chain-reaction (PCR) assay, using C. elegans mtDNA extracts as templates to detect deleted fragments. We optimised a technique to measure accumulated insoluble proteins in C. elegans. We studied the dynamics and sequences of deletion mutations of C. elegans mtDNA, harvested at different ages. We found that with age, mtDNA deletion burden does not increase in C. elegans suggesting that the deletion mechanisms may not be relevant to ageing and are private to C. elegans. We also investigated several genes related to the insulin/IGF pathway as modulators of lifespan in C. elegans which leads to changes in protein aggregation, suggesting that genetic interventions targeting classical ageing pathways can control the accumulation of protein aggregates.

P-275 Transcriptional regulation by vitamin B12 of different metabolic pathways

Gabrielle Giese, Marian Walhout

Program in Systems Biology and Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA 01605, USA.

What we eat affects our metabolism. Our diet contributes to diseases such as diabetes or obesity. Changes in our metabolism can affect gene expression. This gene regulation can happen at the transcription level whereby a metabolite affects a transcription factor that activates or represses the expression of metabolic genes. In this way, metabolism can influence the gene regulatory network, which in turn can influence the metabolic network. We have previously found that bacterially supplied vitamin B12 transcriptionally rewires C. elegans metabolism. For instance, under low vitamin B12 conditions, the short chain fatty acid propionate accumulates and activates the expression of an alternate propionate breakdown pathway, or shunt. The first gene in this pathway, is repressed several hundred fold on diets high in vitamin B12. We recently delineated the mechanism by which propionate activates shunt gene expression. By using an Pacdh-1::GFP transgene, we found that this reporter not only responds to propionate but is affected by vitamin B12 through other mechanisms as well. With the use of forward and reverse genetics, we found that acdh-1 responds to several other metabolic pathways, including the citric acid cycle and the one-carbon cycle. Both of these pathways affect acdh-1 expression through different transcription factors compared to its activation by propionate. Our findings suggest that acdh-1 is controlled by separate metabolic processes through an intricate gene regulatory network, and indicates the multi-functionality of the ACDH-1 enzyme.

P-277 Genetic dissection of neuropeptide cell biology at high and low activity in a defined sensory neuron.

patrick laurent, et al.

Université Libre de Bruxelles, UNI, Laboratory of Neurophysiology, Belgium

Neuropeptides are ubiquitous modulators of behavior and physiology. They are packaged in specialized secretory organelles called dense core vesicles (DCVs) that are released upon neural stimulation. Unlike synaptic vesicles, which can be recycled and refilled close to release sites, DCVs must be replenished by de novo synthesis in the cell body. We dissect DCV cell biology in vivo in a C. elegans sensory neuron whose tonic activity we can control using a natural stimulus. We express fluorescently-tagged neuropeptides and define parameters that describe their sub-cellular distribution. We measure these parameters at high and low neural activity in 187 mutants defective in proteins implicated in membrane traffic, neuroendocrine secretion, and neuronal or synaptic activity. We analyse these data using unsupervised hierarchical clustering methods, and identify 62 groups of genes with similar mutant phenotypes. We explore the function of a subset of these groups. We recapitulate many previous findings, validating our model. We uncover a large battery of proteins involved in recycling DCV membrane proteins, something hitherto unexplored. We show that the unfolded protein response promotes DCV

production, which may contribute to inter-tissue communication of stress. We also find evidence that different mechanisms of priming and exocytosis may operate at high and low neural activity. The work provides a defined framework to dissect DCV biology at varying neural activities.

P-279 syntaxin 7 Promotes Actin Localization During Spermatid Cell Division

Kristin Fenker, Linda Nikolova, Gillian Stanfield

University of Utah

While cell division patterns vary across cell types, in all cases intricate interplay between membrane traffic and the cytoskeleton is essential. We identified a novel function for the gene syntaxin 7 in C. elegans spermatogenesis. Without syx-7, sperm complete most steps of division, including karyokinesis and partitioning of cellular components. However, as they near abscission, F-actin becomes mislocalized and spermatids fail to separate. Strikingly, this defect is limited to spermatocytes that undergo partial cytokinesis at meiosis I; spermatocytes that fully separate at this stage appear to localize actin appropriately and generate functional haploid spermatids. This work suggests distinctive trafficking machinery might modulate cellular division when specific requirements must be met, such as the distinct spatial conformation exhibited by synchronously budding spermatids.

P-281 Autophagy: load-bearer of C. elegans' metabolic changes

Ludovico Martins Alves, Christian Pohl

Buchmann Institute for Molecular Life Sciences, Institute of Biochemistry II, Goethe University Medical School, Max-von-Laue-Strasse 15, 60438, Frankfurt (Main), Germany. pohl@em.uni-frankfurt.de

C. elegans represents an ideal animal model to quantitatively study the autophagic dynamics across multiple stages of development and in response to different sources of stress. Through live imagining and metabolic assays we characterized how autophagy cross talks with mitochondrial maintenance as well as lipid metabolism. First, we observed that dietary changes favorIng propionate metabolism rely on metabolic adaptations induced by autophagy, in a manner independent of previously reported folate or nutritional restriction-induced regulation. Second, since autophagy-induced energy adaptation has been found to be associated with mitochondrial quality control, we tested how the core autophagy factors ATG-7 and ATG-9 as well as the metaloproteases CLPP-1 and YMEL-1 essential for maintenance of mitochondrial respiration, health and lifespan, affect metabolism and aultophagy dynamics. Third, we screened autophagy factors that could regulate the balance between lipogenesis and lipophagy. We found that lipid droplets are not mobilized in response to nutritional restriction in animals compromised for ATG-9 and ATG-18(WIPI1/2). While we observed evidence of drastic metabolic adaptations that require cross-talk between regulatory pathways, we are still actively searching for factors responsible for specific interactions. However, live imaging revealed that autophagy-related regulatory events seem to involve fast membrane reorganization and signalling at time scales much shorter than previously suggested Based on this evidence, we suggest that autophagy plays a major role in both basal and stress metabolism over the course of the C. elegans life cycle and is an essential part of a homeostasis monitoring apparatus.

P-285 Dopaminergic modulation by quercetin: in silico and in vivo evidences

Daiana Avila[1], Willian Salgueiro[1], Fávero Paula[2], Rafaela Rios-Anjos[3], Michael Aschner[4]

[1]*Grupo de Pesquisa em Bioquímica e Toxicologia em Caenorhabditis elegans (GBToxCE), Universidade Federal do Pampa - UNIPAMPA*

[2]*Laboratório de Desenvolvimento e Controle de Qualidade em Medicamentos (LDCQ), Universidade Federal do Pampa*

[3]*Escola de Artes, Ciências e Humanidades, Universidade de São Paulo*

[4]*Department of Molecular Pharmacology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461*

Quercetin is a widely distributed flavonol and has various described biological actions. There have been reports of flavonoid-rich vegetal extracts that can restore neuronal function in diverse model diseases, such as

Parkinson's. Indeed, quercetin alone can act as a neuroprotectant, especially in those diseases linked to impaired dopaminergic neurotransmission. However, little is known about how this flavonoid may interact with the dopaminergic machinery. To study this likely interaction, we exposed the nematode C. elegans to quercetin and observed locomotor behavior by analyzing body bends in NGM off food. After observing behavioral modulation upon quercetin exposure, we determined gene expression of the dopaminergic machinery and then constructed a homology based dopamine transporter protein model to conduct a docking study with quercetin. We have found that quercetin increased body bends off food, as exogenous dopamine did. This modulatory effect was dependent on the presence of the dopamine transporter (dat-1), which was overexpressed, and dopamine receptor 2 (dop-2), which seems to be mandatory for dat-1 overexpression. Hence we postulated that quercetin may act as a dopaminergic modulator by interacting with C. elegans dopamine transporter in silico to modulate the nematode exploratory behavior in vivo. Our data indicates that quercetin may have affinity to the C. elegans DAT-1 homology-based model protein. Once quercetin is a major constituent of many different food sources, this insight over its possible interaction with the dopaminergic machinery may help to establish its potential as an effective natural neuroprotective agent.

P-287 Uniaxial loading induces a scalable switch in cortical actomyosin flow polarization and reveals mechanosensitive components of cell division

Christian Pohl, Deepika Singh, Devang Odedra
Buchmann Institute & Institute of Biochemistry 2, Goethe University, Medical School, Frankfurt, Germany

Force generation in the cell's actomyosin cortex drives cell division, including cortical contractile actomyosin flow. How cortical flow can simultaneously respond to and create forces during cytokinesis is still not well understood. Here we show that under mechanical stress cortical actomyosin flow switches its polarization during cytokinesis in the C. elegans embryo. In unstressed embryos, longitudinal compressive cortical flows contribute to contractile ring formation, while rotational cortical flow is additionally induced in uniaxially loaded embryos. Rotational cortical flow is required for the redistribution of the actomyosin cortex cortical filaments in loaded embryos. Rupture of longitudinally aligned cortical fibres during cortex rotation releases tension, initiates orthogonal compressive longitudinal flow and thereby contributes to furrowing in loaded embryos. A targeted screen for factors required for rotational flow revealed that actomyosin regulators involved in RhoA regulation, cortical polarity and chirality are all required for rotational flow and become essential for cytokinesis under mechanical stress. In sum, our findings extend the current framework of mechanical stress response during cell division and show a directly opposed scaling of orthogonal cortical flows to the amount of mechanical stress

P-289 The Exocyst Complex Promotes Germline Stem Cell Proliferation by Regulating the Trafficking of Notch Receptor in Caenorhabditis elegans

Pushpa Kumari, Harsh Kumar, Sivaram Mylavarapu
Laboratory of Cellular Dynamics, Regional Centre for Biotechnology, NCR Biotech Science Cluster, Faridabad 121001

Notch (Glp-1) is an evolutionary conserved intracellular signaling pathway that controls cell patterning and fate decisions in metazoan development. Misregulation of Notch signaling is associated with developmental defects and diseases including cancer. Studies from Drosophila have reported a pivotal role of vesicular trafficking in activating and regulating Notch signaling. Exocyst, a conserved octameric complex, is required for trafficking of Golgi-derived vesicles and recycling endosomes to distinct sites on the plasma membrane. In C. elegans, the Exocyst has been shown to play role in hypodermal cell migration (Frische et. al, 2007), excretory canal luminogenesis (Armenti et al., 2014) and dendritic branching (Taylor et al., 2015). However its role in germline development has not been demonstrated. We have identified a new role of the Exocyst complex in Notch signaling in Caenorhabditis elegans germline. Partial depletion of Exocyst components by RNAi resulted in reduced number of germline stem cells (GSCs) and low rates of proliferation. Germline stem cell divisions is regulated by the canonical Notch/Delta signaling in C. elegans. Epistasis analysis of Exocyst with notch/glp-1 indicated that Exocyst positively regulates notch/glp-1 function in the germline. Exocyst depleted worms precociously expressed differentiation marker GLD-1 in GSCs and had fewer oocytes displaying delay in

cellularization. All these phenotypes are indicative of low notch/glp-1 activity. We found that the trafficking of Notch/GLP-1 to the plasma membrane of germline stem cells was severely affected upon Exocyst depletion reducing the level of receptors on the membrane. Furthermore, our studies in mammalian cells show that the function of Exocyst complex in trafficking of Notch/GLP-1 receptor is also conserved in mammals. We also find a direct interaction between human Notch receptor and components of the Exocyst complex, providing molecular insight into the role of the Exocyst complex in intracellular trafficking of Notch/GLP-1.

P-291 Overexpression of Werner syndrome helicase extends lifespan and healthspan in C. elegans

Hayley Lees, Sara Maxwell, Lynne Cox, Alison Woollard

Department of Biochemistry, University of Oxford, Oxford OX1 3QU, UK

Progeroid syndromes have yielded informative insights into the mechanisms of human ageing. The causative gene in premature ageing human Werner syndrome (WS) is the dual function WRN helicase/exonuclease that has been implicated in many aspects of DNA metabolism. We have previously shown that WRN is present at a subset of replication foci in normally proliferating cells and that DNA replication fork progression is defective in WS patient-derived cells. While DNA damage and genome instability are predisposing factors for progeroid syndromes and normal ageing, evidence that the genes causative in progeroid syndromes serve a role in preventing ageing is at best only correlative. To test whether WRN can indeed improve ageing outcomes, we have generated transgenic C. elegans worms which overexpress the worm orthologue of WRN, wrn-1. We find that wrn-1 overexpression not only corrects lifespan defects in wrn-1- mutant worms, but that overexpression in a wild type background extends lifespan significantly over than of control wild type animals. Moreover, wrn-1 overexpression results in improved morphology, tissue integrity and movement throughout the life course - the first demonstration that a progeroid-associated gene product is effective in delaying ageing pathologies. Hence we conclude that wrn-1 protects against ageing phenotypes and moreover that normal levels may be limiting for lifespan and health, with significant implications for human ageing.

P-293 IDENTIFICATION OF GENES INVOLVED IN THE INFERTILITY OF INSULIN MUTANTS

Cristina Viera-Osorio, Carlos Lopez-Viso, Sara González-Hernández, Manuel J. Muñoz-Ruiz, Marta Artal-Sanz, et al.

Andalusian Centre of Developmental Biology (CABD). CSIC-Universidad Pablo de Olavide.

The Insulin/Insulin-like growth factor Signalling (IIS) pathway is conserved through evolution in all metazoans including C. elegans. Reduction of the activity of the IIS pathway generates a severe reduction of fertility in all model organisms studied and also in humans. C. elegans is a good organism to study the molecular basis of this infertility. Mutations in the C. elegans insulin receptor daf-2 gene or the phosphatidylinositol 3-kinase age-1 gene generates a severe reduction of fertility. This phenotype can be suppressed by the null mutation of the transcription factor DAF-16/FOXO. The aim of this work is to find C. elegans genes orthologous to human genes whose silencing increases fertility. We have generated a RNAi library containing 6.300 C. elegans genes considered orthologous to humans. With this tool, we screened age-1 mutants to identify conserved genes that regulate infertility in IIS mutants. We found a total of 42 genes, that when inhibited by RNAi significantly increase fertility. Validating the screen, daf-16 is one of the genes that appears as a strong suppressor. We will do a bioinformatics analysis in an attempt to find connections among our selected genes to understand which molecular pathways are related to fertility. In addition, we selected compounds that inhibit the activity of those genes. Those compounds are being tested for their ability to suppress infertility of the IIS muntants. Those inhibitors may have biomedical implications to treat human infertility related to diabetes.

P-295 IDENTIFICATION OF NATURAL EXTRACTS THAT AFFECT GONADS DEVELOPMENTAL ARREST OF INSULIN/IGF MUTANTS.

Carlos López Viso[1], Manuel Alaiz Barragán[2], Ana María Brokate Llanos[1], Andrés Garzón Villar[1], Julio Girón Calle[2], Javier Vioque Peña[2], Manuel Jesús Muñoz Ruiz[1]

[1]*Andalusian Centre for Developmental Biology (CABD), CSIC-Universidad Pablo de Olavide; Department of Molecular Biology and Biochemical Engineering.*
[2]*Fat Institute of Seville (CSIC)*
C. elegans is a suitable organism to study different pathways that have been conserved in both metazoans and humans. In particular, the Insulin/Insulin-like growth factor signalling (IIS) pathway is conserved throughout evolution; and the reduction of its activity produces gonads developmental arrest in C. elegans. Therefore, we have searched for natural extracts that suppress this effect in mutants of IIS pathway. We have performed a screening of hundreds of natural extracts. From those, two different samples partially suppress the gonads developmental arrest in these mutants. Progress in the purification of the active principle will be presented.

P-297 Using C. elegans to investigate natural variation in protein mis-folding and related diseases

Yu Nie[1], Yiru Wang[1, 2], Simon Harvey[1]
[1]*Canterbury Christ Church University*
[2]*Wageningen University*
Polypeptide chains fold correctly into their native states to execute biological functions. Protein misfolding and aggregation nevertheless occur, particularly as organisms age, and this can cause devastating diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD). Numerous C. elegans protein misfolding disease models have been constructed, this has however nearly all been done in a single genotype (N2) of the worm. Only one recent study has investigated a polyglutamine disease model using nematode recombinant inbred lines (RILs), and has demonstrated that natural genetic variation determines susceptibility to the toxicity of polyQ. Here we have looked more systematically at the effect of natural genetic variation on protein misfolding disease using models of AD and PD. AD is associated with extracellular amyloid deposits and intracellular tau. PD is linked to excessive a-synuclein. We have introgressed a human β-amyloid transgene or an α-synuclein transgene from N2 into different nematode genetic backgrounds. A wide range of protein mis-folding disease associated pathology was noted in these AD and PD introgression lines (ILs), for instance, delayed development, defective chemotaxis response, reduced liquid thrash activity, paralysis-like behaviour and deteriorated lipid metabolism especially exposed to neurotoxic nanospheres. The susceptibility of different genetic backgrounds to the introgression varies, and we show this to be independent of the expression level of the pathogenic protein.

P-299 The transcription factors LIN-31 and LIN-1 play a role in toroid formation during morphogenesis of the C. elegans vulva

L.M. Miller[1], A. Hajnal[2]
[1]*Santa Clara Univ, Santa Clara, CA*
[2]*Institute of Molecular Life Science, University of Zurich, Zurich, CH*
The process of morphogenesis--how cells move, change shape, and form complex organs remains one of the biggest mysteries in developmental biology. Vulval morphogenesis in C. elegans involves cell-fate specification, invagination, lumen formation, cell adhesion, cell fusion, cell migration, and cell invasion. While much progress towards understanding the signaling pathways that specify initial vulval cell fates has been made, an understanding of later morphogenetic events and how they are connected to cell fate specification is limited. LIN-31 and LIN-1 are transcription factors (TFs) phosphorylated by MAP kinase (MAPK). We are trying to understand the role that phosphorylation of these TFs plays in vulval development. Interestingly, while phosphorylation-defective LIN-31 or LIN-1 single mutants lack an obvious phenotype, the lin-31; lin-1 double phosphorylation-defective mutants display a 60% egg-laying defective (Egl) phenotype. Using confocal microscopy and an AJM-1::GFP reporter construct, preliminary studies have revealed defects in toroid fusion and alignment in the double phosphorylation-defective mutants. Both LIN-31 and LIN-1 are known to bind the promoter of the lin-39 hox gene. LIN-39 induces the vab-23 gene, which regulates cell fusion and cell guidance genes such as smp-1 semaphorin (Pellegrino et al. 2011). We believe that the morphogenesis defects in the lin-31; lin-1 double phosphorylation-defective mutants support a connection between cell fate determination and execution (morphogenesis). We plan to study the role of LIN-31 and LIN-1 phosphorylation on the regulation of

lin-39 and its downstream targets, including vab-23 and its target genes affecting cell fusion and migration. Our studies support our previous hypothesis that vulval fate specification and morphogenesis are more tightly linked than previously thought. The identification of LIN-31 and LIN-1 as additional late EGFR/RAS/MAPK targets has the potential to shed light on the regulatory network controlling vulval morphogenesis.

P-301 The coiled-coil protein PCMD-1 organizes the assembly of the centrosome matrix

Anna C. Erpf[1], Nadin Memar[1], Ralf Schnabel[2], Mikeladze-Dvali Tamara[1]
[1]*Ludwig-Maximilians-University Munich, Planegg-Martinsried, Germany*
[2]*Technische Universität Braunschweig, Braunschweig, Germany*

Centrosomes, the major microtubule organizing centers of animal cells, are essential for the assembly of a bipolar spindle during mitosis. Spindle defective-5 (SPD-5), the main centrosome matrix protein in C. elegans, forms a tight core around non-mitotic centrioles. To allow for robust nucleation of microtubules at mitotic entry, the centrosome matrix expands isotropically in a Polo Kinase-1 (PLK-1) phosphorylation-dependent manner. However, the molecular basis of the non-mitotic centrosome core formation and what facilitates an organized mitotic SPD-5 expansion into a centrosome with nearly perfect circularity in vivo remains unknown. Here, we present the molecular function of Pericentriolar Matrix Deficient-1 (PCMD-1), a so far uncharacterized centrosome component in C. elegans. We show that the coiled-coil protein PCMD-1 is necessary for an efficient loading of SPD-5 and PLK-1 to the non-mitotic centrosomes of the newly fertilized zygote. Additionally, we show that in absence of PCMD-1 the establishment of the circularity of the mitotic centrosome fails. We propose that PCMD-1 forms a molecular platform, which brings together pericentriolar material (PCM) components and mitotic regulators, allowing for their cross-regulation at mitosis. Thereby PCMD-1 organizes the mitotic PCM and guarantees spindle bipolarity.

Friday, 15 June 2018 - Auditorium - 09:00 – 11:35

Session 5: Morphogenesis

S5-01 The Basement Membrane Toolkit: Looking Outside the Cell

David R. Sherwood
Duke University, Department of Biology, Regeneration Next, Durham NC, 27708 USA

Basement membranes are an ancient form of extracellular matrix that assemble as thin, dense sheets that underlie most tissues. Basement membranes regulate numerous cellular and tissue functions, and defects in basement membrane assembly and composition result in a multitude of human diseases. Despite their importance, we know little about how basement membranes are uniquely constructed, how they grow, how they turnover, and how they regulate so many cell and tissue properties. *C. elegans* is a powerful model to understand basement membrane function as it has single genes encoding most basement membrane matrix components and receptors. We have used CRISPR/Cas-9 genome editing to create a basement membrane toolkit, where we have knocked fluorescent proteins into all major basement membrane matrix components (17 genes) and receptors (13 genes). I will present a brief overview of how we are using the basement membrane toolkit to elucidate the dynamic nature of basement membranes, including the diverse turnover rates of basement membrane components (seconds to hours) and how basement membranes are uniquely constructed on tissues. Finally, I will discuss how the toolkit is revealing specialized, non-sheet functions for BM proteins, such as linking neighboring tissues together--a form of connection that underlies construction of numerous vertebrate tissues, including the blood brain barrier and kidney glomerulus.

S5-02 The Pre-Replication Complex Governs the Invasive Cell Fate of the Caenorhabditis Elegans Anchor Cell in a Replication-Independent Manner

Evelyn Lattmann[1, 2], Ting Deng[1], Vibhu Prasad[1], Charlotte Lambert[1], Michael Daube[1], Ossia Eichhoff[2], Urs Greber[1], Reinhard Dummer[2], Mitch Levesque[2], Alex Hajnal[1], et al.

[1]University of Zurich Institute of Molecular Life Sciences Winterthurerstrasse 190 CH-8057 Zürich Switzerland

[2]University Hospital Zurich Dermatology Wagistrasse 14 CH-8952 Schlieren Switzerland

Insights into the mechanisms of cell invasion, the first step of metastasis formation, holds great promise for the development of new targeted therapies of malignant cancers. Here, we used the Caenorhabditis elegans anchor cell invasion model to gain further knowledge of invasion through basement membranes. By screening the *C. elegans* orthologs of genes highly expressed in invasive human melanoma cells, we identified five pre-replication complex components, mcm-7, cdc-6, cdt-1, orc-2 and orc-5 that regulate basement membrane breaching by the anchor cell. Despite the well-described function of the pre-replication complex in origin of replication licensing for DNA replication in S-phase, we found that mcm-7 regulates invasion in the G0/G1 arrested anchor cell. Furthermore, components of the DNA replication machinery (e.g. GINS complex, cdc-7) are not required for anchor cell invasion, suggesting a DNA replication-independent function of the pre-replication complex. RNAi-mediated knock-down of mcm-7 interferes with invadopodia formation and reduces the expression of the extracellular matrix proteins zmp-1, him-4 and protocadherin cdh-3. Preliminary data suggest that the reduced expression of these pro-invasive genes is a consequence of decreased egl-43L transcription, while mcm-7 RNAi does not affect hlh-2 or fos-1 expression. In the human melanoma cell line A375, knockdown of MCM7 by siRNA causes an accumulation of cells in the G1 phase of the cell cycle with unchanged expression of CCND1. Currently, we are investigating whether pre-replication complex components regulate the invasive behaviour of human melanoma cells. In summary, we propose that components of the pre-replication complex have adopted a replication-independent function to regulate anchor cell invasion.

S5-03 Extrinsic stress triggers actin-based viscoplasticity to drive progressive body axis elongation

Alicia Lardennois[1], Gabriella Pásti[2], Teresa Ferraro[1], Julien Pontabry[2], David Rodriguez[2], Flora Llense[1], Samantha Kim[2], Christelle Gally[2], Michel Labouesse[1, 2]

[1]Sorbonne Université, IBPS, CNRS UMR7622, 7 Quai St-Bernard, 75005 Paris, France

[2]Development and Stem Cells Department, IGBMC – CNRS UMR 7104/ INSERM U964/ Université de Strasbourg, 1 rue Laurent Fries, 67400 Illkirch, France

Body axis elongation represents a fundamental morphogenetic process in development, which involves cell shape changes powered by mechanical forces. Such changes occur through small incremental steps, suggesting the existence of specific mechanisms to stabilize cell shapes and counteract cell elasticity. To understand this we study *C. elegans* embryonic elongation. Our main goal is to understand how the epidermis, an elastic material, acquires progressively its shape through cycles of muscle contractions. To identify the potential morphogenetic lock that would counteract elasticity, we focused on the kinase PAK-1, previously found to mediate a mechanotransduction system downstream of muscle contractions. We performed two screens in a pak-1(Ø) background, from which α-spectrin SPC-1 came out as a strong candidate. We found that spc-1(-) pak-1(-) embryos elongate up to 1.5-fold and then retract to 1-fold in a muscle dependent manner. Another screen in a spc-1(Ø) background identified FHOD-1, a formin with actin bundling properties, whose absence, combined with spc-1 mutation, also induces retraction. To assess the connection between PAK-1, SPC-1 and FHOD-1, we performed rescue experiment and observed partial rescue of the spc-1(-) pak-1(-) retraction phenotype when overexpressing a FHOD-1(ΔFH2/DAD) construct. At the subcellular level, we found that circumferential actin filament bundles are discontinuous and not fully oriented perpendicular to adherens junctions in spc-1(-) pak-1(-) embryos. Strikingly, Priess & Hirsh (1986) found that actin depolymerization induces embryo retraction, suggesting that actin rearrangement could account for the lock counteracting elasticity. To test this idea, we modeled the embryo as a Kelvin-Voigt material experiencing acto-myosin force from the epidermis plus muscle tension. We could predict embryo lengthening by introducing a viscoplastic component in the system, which we propose corresponds to actin shortening.

Altogether our data identify a cellular network that confers mechanical plasticity to stabilize cell shapes during morphogenesis.

S5-04 Contractile ring-dependent cytoplasmic partitioning in non-mitotic germ cells

Chelsea Maniscalco[1], Jeremy Nance[1, 2]

[1]*Helen L. and Martin S. Kimmel Center for Biology and Medicine at the Skirball Institute of Biomolecular Medicine, NYU School of Medicine, New York, New York 10016, USA*

[2]*Department of Cell Biology, NYU School of Medicine, New York, New York 10016, USA*

Cells such as spermatocytes and erythroblasts form large compartments that are used to eliminate a subset of cellular components. We recently showed that *C. elegans* primordial germ cells (PGCs) change shape autonomously to form large organelle-rich lobes. PGC lobes are cannibalized by neighboring cells, shrinking PGC volume and eliminating most of their mitochondria. Using time-lapse fluorescence microscopy, we observed that PGC lobes form when the nucleus migrates to one side of the cell and the cell constricts at its equator into two halves – the cell body (containing the nucleus and minimal cytoplasm) and the lobe (enriched in mitochondria). Because lobe formation resembles an incomplete cytokinesis, we tested whether lobe formation occurs through the assembly and constriction of a contractile ring, even though PGCs are arrested in interphase. Contractile ring components in dividing cells include actin filaments, the motor protein non-muscle myosin, the cross-linking protein anillin, and membrane-associated septin filaments. By expressing fluorescently tagged non-muscle myosin specifically in the PGCs and co-staining with antibodies recognizing other contractile ring components, we observed that a ring containing non-muscle myosin, anillin, and septin localizes at the lobe neck. To test if ring closure is required for lobe formation, we used temperature-sensitive mutations to acutely block the function of two genes essential for contractile ring formation – nmy-2/non-muscle myosin and the F-actin nucleator cyk-1/formin. Inactivation of either component blocked lobe formation. By contrast, acute inactivation of components of the Centralspindlin complex (cyk-4/MgcRacGAP and zen-4/MKLP1), which localizes to the mitotic spindle in dividing cells and dictates the position of the contractile ring, had no effect on PGC lobe formation. Our findings suggest that an interphase contractile ring, which is assembled and positioned independently of the Centralspindlin complex, constricts to partition the PGC cell body from its lobe, allowing PGCs to remodel their size and contents.

S5-05 Building and shaping seamless tubes: repurposing a cell-cell fusogen for membrane trafficking

Meera Sundaram

University of Pennsylvania

Cell-cell fusogens of the Fusexin structural family mediate membrane merging during viral infection, fertilization, and formation of syncytial tissues. These types of membrane merging events initiate on the exoplasmic (non-cytosolic) side of a cell's plasma membrane, and fuse two separate cells into one. Within an individual cell, some membrane trafficking events, including endocytic scission, also involve membrane merging that initiates on the exoplasmic side of the membrane, but how such fusion and fission occurs is not well understood. We discovered that the *C. elegans* Fusexin protein AFF-1 is required for endocytic scission and apically-directed trafficking during morphogenesis of the excretory duct tube, a unicellular seamless tube. This work expands the potential roles of cell-cell fusogens to intracellular membrane trafficking and suggests a new way to think about the basic process of endocytic scission: that in addition to relying on proteins like dynamin that assemble on the cytosolic side and constrict and pinch the membrane, scission may utilize mechanisms similar to those involved in cell and vesicle fusion, where interactions between transmembrane fusogens help pull membranes into very close proximity for merging.

S5-06 Activation of Aurora A kinase by TPXL-1 clears contractile ring proteins from the cell poles during cytokinesis

Sriyash Mangal[1], Jennifer Sacher[1], Taekyung Kim[2], Daniel Sampaio Osório[3], Fumio Motegi[4], Ana Carvalho[3], Karen Oegema[2], Esther Zanin[1]

[1]Center for Integrated Protein Science, Department Biology II, Ludwig-Maximilians University Munich, Planegg-Martinsried, Germany

[2]Department of Cellular and Molecular Medicine, Ludwig Institute for Cancer Research, University of California, San Diego, La Jolla, CA

[3]Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

[4]Research Link, National University of Singapore, Singapore

During cytokinesis a contractile actin-myosin ring splits the mother cell physically into two daughter cells. Two concurrent signals from the mitotic spindle direct the assembly of the contractile ring at the cell equator in anaphase. A stimulatory signal promotes contractility at the cell equator and an inhibitory signal from the microtubule asters blocks the accumulation of contractile ring components at the cell poles. Although the presence of the inhibitory signal has been demonstrated in many different organisms the molecular identity of the signal was unknown. To identify the inhibitory signal from the mitotic asters during cytokinesis, we developed a microscopy-based assay in the *C. elegans* embryo. Using our assay, we find that TPXL-1, the homologue of human TPX2, is required for clearing the contractile ring component anillin from the cell poles. In tpxl-1 mutant embryos the mitotic spindle is short. To distinguish whether the short spindle or tpxl-1 depletion itself causes defects in anillin clearing, we restored spindle size by depleting HCP-4, a component of the kinetochore. We find that although in hcp-4;tpxl-1 mutant embryos spindle size is normal, anillin clearing from the poles is still defective, indicating that TPXL-1 has a direct role in this process. TPXL-1 binds and activates aurora A kinase and localizes to the centrosome and the astral microtubules. Finally we tested whether clearing of anillin depends on the ability of TPXL-1 to activate aurora A kinase. We find that wild-type TPXL-1, but not aurora A-binding defective TPXL-1, supports anillin clearing from the cell poles. In summary, our work identifies TPXL-1 and aurora A kinase as the first molecular components of the aster-based signal and it suggests that aurora A kinase activation by TPXL-1 is essential for the removal of contractile ring components from the cell poles during cytokinesis.

S5-07 Microtubule Dynamics Scale with Cell Size to Set Spindle Length and Assembly Timing

Benjamin Lacroix[1], Gaëlle Letort[2], Laras Pitayu[1], Jeremy Sallé[1], Julie Canman[3], Nicolas Minc[1], François Nedelec[4], Julien Dumont[1]

[1]Institut Jacques Monod, CNRS, UMR 7592, University Paris Diderot, Sorbonne Paris Cité, F-75205, Paris, France.

[2]Institut Curie, Mines Paris Tech, Inserm, U900, PSL Research University, F-75005, Paris, France.

[3]Columbia University Medical Center, Department of Pathology and Cell Biology, New York, NY 10032, USA.

[4]Cell Biology and Biophysics Unit, European Molecular Biology Laboratory, 69117 Heidelberg, Germany.

Successive cell divisions during embryonic cleavage create increasingly smaller cells, so intracellular structures must adapt their size to remain functional. Mitotic spindle size correlates with cell size, but the mechanisms for this scaling are unclear. Using live cell imaging, we analyzed spindle scaling during embryo cleavage in the nematode Caenorhabditis elegans and sea urchin Paracentrotus lividus. We reveal a conserved scaling mechanism, where the growth rate of spindle microtubules scales with cell volume, which explains spindle shortening. Spindle assembly timing is however constant throughout successive divisions. Analyses in silico suggest that controlling the microtubule growth rate is sufficient to scale spindle length and maintain a constant assembly timing. We further tested our in silico predictions to demonstrate that modulating cell volume or microtubule growth rate in vivo induces a proportional spindle size change. Our results suggest that scalability of the microtubule growth rate when cell size varies could prevent embryonic aneuploidy.

S5-08 *C. elegans* blastomeres clear the corpse of the second polar body by LC3-associated phagocytosis

Gholamreza Fazeli, Maurice Stetter, Jaime Lisack, Ann Wehman
University of Würzburg

To understand how undifferentiated pluripotent cells cope with cell corpses, we examined the clearance of polar bodies born during female meiosis. We found that polar bodies lose membrane integrity and expose phosphatidylserine in Caenorhabditis elegans. Polar body signaling recruits engulfment receptors to the plasma membrane of embryonic blastomeres using the PI3Kinase VPS-34, RAB-5 GTPase, and the sorting nexin SNX-6. The second polar body is then phagocytosed using receptor-mediated engulfment pathways dependent on the Rac1 ortholog CED-10, but undergoes non-apoptotic programmed cell death independent of engulfment. RAB-7 GTPase is required for lysosome recruitment to the polar body phagosome, while LC3 lipidation is required for degradation of the corpse membrane after lysosome fusion. The polar body phagolysosome vesiculates in an mTOR- and ARL-8-dependent manner, which assists its timely degradation. Thus, we established a genetic model to study clearance by LC3-associated phagocytosis and reveal insights into the mechanisms of phagosome maturation and degradation.

Friday, 15 June 2018 - Auditorium - 13:00 – 15:20

Session 6: Evolution & Natural Variation

S6-01 Wild *C. elegans*

Lise Frézal[1, 2], Aurélien Richaud[1], Eric Miska[2], Marie-Anne Félix[1]
[1]*IBENS, Paris, France.*
[2]*Gurdon Institute, Cambridge, UK.*

The nematode *C. elegans* is found proliferating and feeding in decomposing vegetal matter. We will report on wild collections of *C. elegans* and Caenorhabditis species, and on their associated organisms. Following local populations of *C. elegans*, we found that several genotypes co-existed in a given location over several years, without effective recombination. Insights into non-standard heredity in *C. elegans* result from two lines of work. First, the mortal germ line (Mrt) phenotype was defined as a multigenerational phenotype whereby a selfing lineage becomes sterile after several generations, implying multigenerational memory [1]. Second, certain RNA interference (RNAi) effects are heritable over several generations in the absence of the initial trigger [2]. Both lines of work converged when the subset of Mrt mutants that are heat-sensitive were found to closely correspond to mutants defective in the RNAi-inheritance machinery, including histone modifiers [3]. We report the surprising finding that several *C. elegans* wild isolates display a heat-sensitive mortal germline phenotype in laboratory conditions: upon chronic exposure to higher temperatures such as 25°C, lines reproducibly become sterile after several generations. This phenomenon is reversible as it can be suppressed by temperature alternations at each generation, suggesting a non-genetic basis for the sterility. We focused on determining the genetic basis of the quantitative variation found among wild isolates, using 1) laboratory crosses; 2) association mapping. The two approaches yielded several candidate regions. After further recombinant mapping and genome editing, we identified the two major causal loci in 1) as a rare polymorphism in the set-24 gene, encoding a SET- and SPK-domain protein and in 2) as a common polymorphism in the morc-1 gene [4]. We conclude that *C. elegans* natural populations may harbor natural genetic variation in epigenetic inheritance phenomena.

1. Ahmed & Hodgkin 2000, Smelick & Ahmed 2005
2. Grishok et al. 2000, Vastenhouw et al. 2006, Alcazar. et al. 2008.
3. Katz et al. 2009, Xiao et al. 2011, Buckley et al. 2012.
4. Spracklin et al. 2017, Weiser et al. 2017.

S6-02 Small peptide mediated self-recognition prevents cannibalism in predatory nematodes

James Lightfoot, Martin Wilecki, Christian Roedelsperger, Eduardo Moreno, Ralf Sommer
Max-Planck Institute for Developmental Biology, Max-Planck Ring 9, 72076 Tübingen, Germany

Self-recognition is observed abundantly throughout the natural world regulating diverse biological processes. Although ubiquitous, often little is known about the associated molecular mechanisms and despite the prevalence of nematodes in nearly every ecological niche and the pre-eminence of Caenorhabditis elegans as a model organism, evidence of self-recognition has thus far never been described in nematodes. Here we investigate the predatory nematode Pristionchus pacificus and through interactions with its prey, reveal a self-recognition mechanism acting on the nematode surface, capable of distinguishing self-progeny from even closely related strains. We identified a key component of the self-recognition machinery located within a region of minimal recombination activity and as such, developed a novel method of inducing informative recombination events via CRISPR/Cas9. Using this system, we have successfully identified the small peptide SELF-1 as a major component of self-recognition, which is expressed in all hypodermal cells at all stages of the life-cycle. SELF-1 is composed of an invariant domain including a signal peptide and a hyper-variable C-terminus. Comparative analysis of this region reveals extreme variability in sequence, length and also self-1 copy number. Disruptions to the hyper-variable region including even a single amino acid substitution were sufficient to eliminate protection from predation. Thus, the self-recognition system identified in P. pacificus enables this nematode to avoid cannibalism, while promoting the killing of competing nematodes.

S6-03 Dissecting the sources of phenotypic variation among genetically identical individuals growing in the same environment

Mirko Francesconi[1, 2], Marcos Perez[1, 2], Ben Lehner[1, 2, 3]
[1]*Systems Biology Program, Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology (BIST) Dr. Aiguader 88, 08003 Barcelona, Spain*
[2]*Universitat Pompeu Fabra (UPF), Dr. Aiguader 88, 08003 Barcelona, Spain.*
[3]*Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Spain*

Genetically identical individuals growing in the same environment often show substantial phenotypic variation. The reasons for this phenotypic variation are usually unknown. We found that isogenic *C. elegans* growing in the same environment differ in developmental speed, including the relative developmental rate of the soma and the germline (soma-germline heterochrony), fecundity and many other fitness important traits such as size and resistance to early starvation. We found maternal age to be the major determinant of this phenotypic variation in with progeny of young mothers being surprisingly impaired in size, developmental speed, starvation resistance and fecundity, and they also show different soma-germline heterochrony. Changes in maternal provisioning of yolk to the embryo, which increases with age, explain many of these phenotypic differences but do not explain differences in soma-germline heterochrony and fecundity(1). We now have obtained preliminary results showing that parental exposure to pheromone also induces phenotypic differences in the offspring, including soma-germline heterochrony and fecundity, which are not explained by a change in yolk provisioning.
References 1 Perez, M. F., Francesconi, M., Hidalgo-Carcedo, C. & Lehner, B. Maternal age generates phenotypic variation in Caenorhabditis elegans. Nature 552, 106-109, doi:10.1038/nature25012. Epub 2017 Nov 29. (2017).

S6-04 Molecular identification of vulval developmental defects in the non-model nematode Oscheius tipulae via mapping-by-sequencing

Amhed Missael Vargas Velazquez, Fabrice Besnard, Clement Dubois, Marie-Anne Felix
Ecole Normale Supérieure, PSL Research University, CNRS, Inserm, Institut de Biologie de l'Ecole Normale Supérieure, 75005 Paris, France

Oscheius tipulae is a common free-living nematode, phylogenetically closer to the model species Caenorhabditis elegans than the outgroup Pristionchus pacificus. This species is thus enlightening for

comparative genetics, developmental and evolutionary studies despite not being a model organism. In model species with fully assembled and annotated genomes, mapping-by-sequencing has become a standard method to map and identify phenotype-causing mutations. Candidate variants are pinpointed using a cross to a divergent mapping strain and sequencing of a pool of mutant segregants. Here we show that we can apply the mapping-by-sequencing approach with a draft genome assembly of O. tipulae to identify mutations responsible for vulval and other morphological defects. The only Vulvaless mutant we found corresponds to a cis-regulatory deletion in the lin-3 gene. Combining single-molecule FISH, laser ablation and CRISPR-Cas9 deletion results, we demonstrate the role of LIN-3 in vulval induction by the anchor cell in O. tipulae. In contrast, mutants with an excess of vulva induction correspond unexpectedly to the plexin/semaphorin pathway, a signaling pathway not previously found in *C. elegans* vulva development screens. We will present the analysis of these mutants and several others (Wnt pathway, etc.). Our success suggests that a draft assembly of a non-model organism is sufficient to perform mapping-by-sequencing thus bringing non-model species firmly into genomics-enabled science, and providing tools to investigate the evolution of developmental mechanisms.

S6-05 There is more to genetic variation than you think: from gene mapping to complex perturbation analyses

Jan Kammenga

Laboratory of Nematology, Wageningen University, Droevendaalsesteeg 1, 6708PB, The Netherlands.

Genetic pathway analyses has been an important realm in *C. elegans* research with the canonical strain Bristol N2 as the main carrier of these investigations. However, Bristol N2 is not alone anymore. Over the past decade and a half we have witnessed a spectacular rise in worm studies from different labs going beyond N2 by embracing the concept of genetic variation. One of the main messages coming out from these studies is that mutations in pathways seldom yield consistent phenotypes across the richness of strain diversity. Mutant phenotypes in Bristol N2 are a small blip in the phenotypic spectrum of natural variation. This implies that phenotypic effects of mutations and pathways are affected by natural genetic modifiers. I will illustrate the value of finding the natural genetic modifiers of mutations based on gene mapping across different strains affecting genetic pathways. But finding modifier genes is the tip of the iceberg. We have investigated the full genetic architecture of phenotypes and examples will be given for the let-60 Ras pathway. This allows for detection of modifier genes that would not have been detected using mutant screens in Bristol N2. Finally, I will explore how diversity between strains in combination with transcriptome profiling can be used to analyse complex perturbations resulting from environmental transitions.

S6-06 Genome sequence and evolution of the asexual nematode Diploscapter pachys

Kristin Gunsalus[1], Helene Fradin[1, 2], Karin Kiontke[1], Ryan Baugh[2], Fabio Piano[1], David Fitch[1], et al.

[1]*New York University*

[2]*Duke University*

Asexual reproduction has arisen independently multiple times in the phylum Nematoda and requires numerous modifications of the reproductive program to produce a viable diploid zygote without fertilization of the oocyte. In the absence of genetic exchange, the longevity of asexual clades may be correlated with the maintenance of heterozygosity by mechanisms that rearrange genomes and reduce recombination. Asexual species thus provide an opportunity to gain insight into the relationship between genome architecture, molecular networks, and cellular and developmental processes. We have sequenced the genome of the parthenogenetic nematode Diploscapter pachys, which belongs to a long-lived clade of asexual nematodes closely related to *C. elegans*. While most asexual lineages are short-lived, we estimate from sequence divergence that this clade has persisted for at least 18M years. Strikingly, D. pachys and its close relatives harbor only a single pair of large chromosomes We show that the D. pachys genome arose from complete fusion of ancestral chromosomes and lacks typical nematode telomeres and telomere maintenance genes. The genome sequence reveals extensive rearrangement among neighboring ancestral regions, with high

heterozygosity across most regions. Homozygosity is largely concentrated to one region, potentially reflecting asynchronous fusion events, which we propose is maintained by gene conversion. Cell-biological and molecular evidence is consistent with the absence of key features of meiosis I, including synapsis and recombination. The temporal sequence and causal relationships of molecular events leading to chromosomal fusion, altered meiosis, and egg autoactivation are yet to be determined. Elucidating the nature of these changes would provide a deeper understanding of the ways in which animals can modify their reproductive programs during evolution.

S6-07 Single-Molecule Real Time sequencing reveals large structural variants in natural isolates of *C. elegans*

Cristian Riccio[1, 2], Martin Hemberg[1, 2], Eric Miska[1, 2]

[1]*Wellcome Trust Sanger Institute*

[2]*Wellcome Trust/CRUK Gurdon Institute*

C. elegans is the first metazoan that had its genome sequenced. The millions that it cost to sequence it were worth it. The reference genome now allows us to better understand the nematode biology. Through gene predictions, biological functions can be inferred. The reference is necessary in gene expression analysis, CRISPR screens, etc. However, to have a broader understanding of *C. elegans* biology, it is necessary to have access to the whole genome information of other wild isolates. We have used third generation sequencing technologies such as PacBio and Nanopore to sequence a 16 wild isolates of *C. elegans*. The assemblies contain about 100 contigs and have an N50 of 2 MB. Compared to the Wormbase reference, our assemblies have on average an additional 2 megabases. Initial analyses have revealed thousands of structural variants in each strain, including retrotransposition events. Combined with short-read high-quality sequencing, the assemblies contain few errors. This data will allow us to better understand large scale events in genomes as well the evolution of *C. elegans* wild isolates.

Friday, 14 June 2018 – Port Vell Room - 15:30 – 18:00

Poster session II

P-02 Force transmission between three tissues controls planar polarity establishment and embryonic morphogenesis

Ghislain Gillard[1], Ophélie Nicolle[1], Thibault Brugière[1], Sylvain Prigent[2], Mathieu Pinot[1], Grégoire Michaux[1]

[1]*IGDR, Rennes, France*

[2]*Biogeneouest, Rennes, France*

Tissues from different developmental origins interact to achieve coordinated morphogenesis at the level of a whole organism. Accordingly C. elegans embryonic elongation is driven by cell shape changes controlled by actomyosin dynamics in the lateral epidermis and by muscle contractions. But how epidermal cell shape changes and muscle contractions are coordinated is not known. We found that a mechanical force generated by muscle contractions and relayed by molecular tendons in the dorsal and ventral epidermis establishes a newly identified planar polarity of the apical PAR module in the lateral epidermis. The planar polarised PAR module then controls actin planar organisation, thus determining the orientation of tension generated by actomyosin. This tension controls the orientation of cell shape changes which finally determine the elongation axis of the whole embryo. This inter-tissue mechanotransduction pathway therefore contributes to coordinate the morphogenesis of the whole embryo.

P-04 Interacting partners of BLMP-1 as a transcriptional activator in C. elegans

Hei Tung Michelle Fong, Takao Inoue, Thilo Hagen

Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, 117597, Singapore

In the Caenorhabditis elegans, BLMP-1 is the ortholog of the human zinc finger and SET domain-containing transcriptional repressor PRDM1/BLIMP1. PRDM1/BLIMP1 has been widely studied as a transcriptional repressor in many species including humans and mice. Interestingly, we found that in C. elegans, BLMP-1 acts as a direct transcriptional activator of bed-3, a gene required for molting and vulval development. This provides the first evidence that BLMP-1 can act as a transcriptional activator in C. elegans. To study the role of BLMP-1 as a transcriptional activator in C. elegans, we performed a small scale RNAi screen using qPCR and bed-3::gfp reporter, and identified three possible interacting partners of BLMP-1: F58A3.1/LDB-1, F25H8.2 and ZK1128.5/HAM-3. To further confirm that the three proteins are interacting with BLMP-1 to positively regulate bed-3, we tested if the three proteins are acting on the region of the BLMP-1 binding sites, i.e. the enhancer fragment SF2-9 on the bed-3 gene. We found that the SF2-9::gfp reporter was significantly down-regulated only upon RNAi treatment against F58A3.1/ldb-1 and ZK1128.5/ham-3, but not F25H8.2. We also confirmed that F58A3.1/LDB-1, ZK1128.5/HAM-3 and BLMP-1 physically interacts by performing co-immunoprepitation in HEK293T cells using the human orthologs of three proteins. These results strongly suggest that F58A3.1/LDB-1 and ZK1128.5/HAM-3 interacts with BLMP-1 in the positive regulation of bed-3, providing us a better understanding of how BLMP-1 functions as a transcriptional activator.

P-06 Chryseobacterium nematophagum: A novel matrix digesting bacilli with an eclectic nematode diet.

Antony Page[1], Marie-Anne Felix[2], Mark Roberts[1]

[1]*University of Glasgow, Glasgow*

[2]*Ecole Normale Supérieure, Paris*

Nematode killing bacilli were isolated from rotten fruit in association with Caenorhabditis briggsae in France and India. These bacteria belong to the Chryseobacterium genus (Golden bacteria) and represent a new species which we have named Chryseobacterium nematophagum. These bacilli are oxidase-positive, gram-negative rods that exhibit unusual gliding motility and gelatinase activity. C. elegans are attracted to and eat these bacteria, however within 3 hours of ingestion the bacilli have degraded the pharyngeal lining and entered the anterior body cavity killing the host. Within 24 hours the internal contents of the worms are digested followed by the final digestion of the remaining cuticle. These bacilli will also infect and kill bacteriverous free-living (L1-L3) stages of all tested parasitic nematodes including the important veterinary trichostrongylids such as Haemonchus contortus and Ostertagia ostertagi. The bacilli exhibit potent collagen digesting properties and genome sequencing has identified key collagenase and chitinase enzymes that may represent virulence factors. The detailed characterization of this nematode pathogen will be discussed.

P-10 The Small GTPase RAC1/CED-10 Is Essential in Maintaining Dopaminergic Neuron Function and Survival Againstα-Synuclein-Induced Toxicit

Esther Dalfo, et al.

Faculty of Medicine/Universitat de Vic-Universitat Central de Catalunya

Department of Biochemistry and Molecular Biology, Institut de Neurociències, Faculty of Medicine, M2, Universitat Autònoma de Barcelona (UAB), Bellaterra Campus, Cerdanyola del Vallés, Barcelona, Spain

Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL 35487, USA

Department of Pathology and Experimental Therapeutics, Bellvitge University Hospital-IDIBELL, 08028 L'Hospitalet de Llobregat, Spain

Institute of Biomedicine of the University of Barcelona (IBUB), Barcelona 08908, Spain

Center of Regenerative Medicine in Barcelona (CMRB), Center for Networked Biomedical Research on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Hospital Duran i Reynals, 08908 L'Hospitalet de Llobregat, Spain

Buck Institute for Research on Aging, 8001 Redwood Boulevard, Novato, CA 94945, USA

Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío/CSIC/ Universidad de Sevilla, 41013 Sevilla, Spai
Laboratory of Parkinson Disease and Other Neurodegenerative Movement Disorders, Department of Neurology: Clinical and Experimental Research, IDIBAPS–Hospital Clínic de Barcelona, 08036 Barcelona, Spain 8
Neurodegenerative Diseases Research Group, Vall d'Hebron Research Institute-Center for Networked Biomedical Research on Neurodegenerative Diseases (CIBERNED), 08035 Barcelona, Spain
Parkinson's disease is associated with intracellularα-synuclein accumulation and ventral midbrain dopaminergic neuronal death in theSubstantia Nigraof brain patients. The Rho GTPase pathway, mainly linking surface receptors to the organization of the actin and microtubule cytoskeletons, has been suggested to participate to Parkinson's disease pathogenesis. Nevertheless, its exact contribution remains obscure. To unveil the participation of the Rho GTPase family to the molecular pathogenesis of Parkinson's disease, we first usedC elegansto demonstrate the role of the small GTPase RAC1(ced-10in the worm) in maintaining dopaminergic function and survival in the presence of alpha-synuclein. In addition,ced-10mutant worms determined an increase of alpha-synuclein inclusions in comparison to control worms as well as an increase in autophagic vesicles. We then used a human neuroblastoma cells (M17) stably over-expressing alpha-synuclein and found thatRAC1function decreased the amount of amyloidogenic alpha-synuclein. Further, by using dopaminergic neurons derived from patients of familial LRRK2-Parkinson's disease we report that human RAC1 activity is essential in the regulation of dopaminergic cell death, alpha-synuclein accumulation, participates in neurite arborization and modulates autophagy. Thus, we determined for the first time tha tRAC1/ ced-10participates in Parkinson's disease associated pathogenesis and establishedRAC1/ced-10as a new candidate for further investigation of Parkinson's disease associated mechanisms, mainly focused on dopaminergic function and survival againstα-synuclein-induced toxicity.

P-12 Lamin plays with SUN

Lenka Hůlková[1], Jana Rohožková[1], Pavel Hozák[1, 2]
[1]*Institute of Molecular Genetics AS CR, v.v.i. division BIOCEV, Průmyslová 595, Vestec, Czech Republic*
[2]*Institute of Molecular Genetics AS CR, v.v.i., Vídeňská 1083, Prague, Czech Republic*
Intermediate filaments (IFs) are cytoskeletal components composed of a family of related proteins sharing common structural and sequence features. Except cytoplasmic IFs, we know about nuclear IFs - lamins, which belongs to this protein family (as type V), and are a main component of nuclear lamina. Lamins are organized at the nuclear periphery and have many roles, such as maintenance of nuclear shape, regulation of gene expression, transcription, DNA replication, segregation of chromosome, meiosis and apoptosis. Mutations in lamin genes have dramatic effect on their function causing pathological phenotype (i.e. Emery-Dreifuss Muscular Dystrophy or Hutchinson-Gilford Progeria Syndrome). We identified novel function of lamin in process of gametogenesis in Caenorhabditis elegans. This model organism offers an advantage of only one evolutionary conserved lamin protein (CeLMN) compared to mouse or human cell lines. Our project focuses on the molecular mechanisms and regulation of chromosomal dynamics during gametogenesis. We study the SUN/KASH complex, which is important in the process of chromosome pairing at early stages of meiosis I. Interestingly, we discovered that some dedicated mutations in CeLMN gene have affects SUN-1 aggregation at the nuclear periphery during meiotic prophase I. Additionally, we identified new protein DEB-1, which is involved in the stabilization of nuclear periphery and association of SUN-1 with chromosome termini. We suggest that CeLMN, SUN and DEB-1 proteins form complex, which is involved in process of chromosome pairing in prophase I.

P-14 Quantitative phenotyping and modeling identifies key behavioral rules underlying C. elegans aggregation

S Serena Ding[1, 2], Linus Schumacher[3, 4], Robert Endres[3, 4], Andre Brown[1, 2]
[1]*MRC London Institute of Medical Sciences, UK*
[2]*Institute of Clinical Sciences, Imperial College London, UK*

[3]*Department of Life Sciences, Imperial College London, UK*
[4]*Centre for Integrative Systems Biology and Bioinformatics, Imperial College London, UK*
In complex biological systems, simple individual-level behavioral rules can give rise to emergent group-level behavior. While such collective behavior has been well studied in cells and larger organisms, the mesoscopic scale is less understood. Here, we investigate collective feeding in the roundworm C. elegans at this intermediate scale, and use quantitative phenotyping and agent-based modeling to identify behavioral rules underlying aggregation. We use fluorescent multi-worm tracking to quantify aggregation behavior in terms of individual dynamics and population-level statistics. Based on our quantification, we use agent-based simulations and approximate Bayesian inference to identify two key behavioral rules that give rise to stable aggregation, namely cluster-edge reversals and density-dependent switch between crawling speeds.

P-16 A GFP::OB fold gene promoter fusion screen aim to identify cytosolic sensor receptor of viral immunity reveal the involvement of DNA licensing pathway components

Priyanka Mishra, Sheliza Shivji, Megan Cornell, Brandon Kong, Victoria Kooner, Albert Luu, Gabriel Lim, Frederic Pio
Molecular Biology and Biochemistry Department, Simon Fraser University, Burnaby-V5A1S6, British Columbia, Canada
The Orsay virus/C. elegans model system provides an opportunity to study receptors mediated innate immune pathways. Two pathways have been reported but in C. elegans only TOL-1 with other downstream pathway components viz. TRF-1, PIK-1, and IKB-1 exist. In fact, the cytoplasmic sensors receptors found only in human (HIN200/IFI16/AIM2) has not been found. The binding of the viral nucleic-acids to OB fold domain of these receptors creates a signalosome complex called inflammasome that activates IL-β. While, we have shown the existence of human PRR homologues in C. elegans the downstream pathway components STING, TBK-1, cytokine, interleukin, NFkB, interferons, IRF-3 are absent. This indicates that worm may use either novel(s) RNAi, NFkB and TLR independent pathway. As such, the STAT pathway activated by Orsay virus suggest that a cytoplasmic sensor receptor could exist upstream of the pathway. To identify these cytoplasmic receptors we developed a functional screen where (i) C. elegans PRRs should contain an OB fold to recognize viral nucleic-acids (ii) Transgenic line containing GFP::OB fold gene promoter fusion should display a differential pattern of gene expression in infected vs. uninfected transgenic lines. Using confocal microscopy on each transgene we found that the spatial and temporal GFP expression was different for two infected transgenic lines. We also identified differential expression in the pharynx for gene specific promoters that belong to the MCM gene family. These helicases unwinds the DNA template prior replication a necessary step of DNA licensing. It suggests that Orsay may hijack some of the components of host DNA machinery to replicate its nucleic-acids. It is consistent with other studies involving the mcm complex during replication of HIV, adenovirus, influenza, and cytomegalovirus. Abbreviations+Keywords: C. elegans, Orsay virus, PRR: Pattern Recognition Receptors, Cytosolic sensor receptors, OB fold: Oligonucleotide Binding fold, GFP: Green Fluorescent Protein, MCM: Mini Chromosome Maintenance Complex

P-18 Regulation of DNA repair pathways to ensure gamete quality

Erika Rosenkranse, Carolyn Turcotte, Julia Laibach, Aidan Nowakowski, Richard Monsky, Nicolas Andrews, Paula Checchi
Marist College
Meiotic recombination requires the formation and repair of genome-wide, programmed double-stranded breaks (DSBs). Repair of meiotic DSBs requires homologous recombination (HR), an error-free repair pathway required for crossover formation. Accordingly, error-prone pathways such as non-homologous end joining (NHEJ) are not favored during meiosis due to their propensity for generating mutations and their inability to form chiasmata. How error prone repair pathways are suppressed during meiosis is not well understood. Here, we demonstrate a role of for Mi2, the core ATPase subunit of the NuRD chromatin remodeling complex, in coordinating the repair of DSBs and maintaining genomic stability through multiple mechanisms. Our data reveal that the conserved Mi2 homologs CHD-3 and LET-418 promote HR, though in their absence, NHEJ is engaged as a secondary DNA repair mechanism to prevent persisting damage in gametes. In support of this, our findings indicate that a population of DSBs are repaired

via NHEJ in situations with compromised LET-418 activity. Though HR mechanisms remain partially intact in let-418 mutants, their germ lines possess multiple defects including increased corpses and presence of structurally abnormal chromosomes in oocytes. These data are corroborated by molecular evidence wherein let-418 mutants have elevated expression of several NHEJ components, indicating a role for NuRD in the transcriptional regulation of repair genes during meiosis. Intriguing, our data also reveal that loss of LET-418 leads to upregulation HR machinery, which we attribute to increased genomic stress in mitotically dividing germ cells, and we are currently investigating the causes and consequences of this. As these genes involved in these processes are highly conserved throughout eukaryotes, our findings have implications for understanding how Mi2 may contribute to the prevention of human disease states such as infertility and cancer.

P-20 Unraveling the molecular mechanisms controlling monoaminergic neurons development through an RNAi screen

Ángela Jimeno-Martín, Miren Maicas Irigarai, Rebeca B. Ruiz, Nuria Flames Bonilla
Institute of Biomedicine of Valencia (IBV-CSIC)

Monoamines comprise a group of neurotransmitters that include serotonin, dopamine, adrenaline, noradrenaline, tyramine and octopamine. Monoamines are involved in the regulation of a plethora of behaviors and their dysfunction is linked to several pathological conditions such as Parkinson disease, depression or bipolar disorder. Each monoaminergic neuron subtype expresses a different subset of enzymes to synthesize the corresponding monoamine; however, they all share the expression of the vesicular monoamine transporter (cat-1) that packages the neurotransmitter. To further understand how the monoaminergic system develops, we take advantage of the phylogenetically conservation of this neuronal population to study it in the nematode C.elegans. In this nematode, the monoaminergic system is composed by 11 different neuron classes: the serotonergic NSM, ADF, HSN, AIM and RIH neurons, the dopaminergic ADE, PDE, CEPV and CEPD, the tyraminergic RIM and the octopaminergic RIC. We benefit from the amenability of RNA-interference (RNAi) technology to test what is the effect of knocking down each C. elegans transcription factor (TF) gene (a total of 876) in monoaminergic specification. In our screen we combined three reporters to increase the information retrieved: the pan-monoaminergic marker cat-1::gfp, a serotonergic marker (tph-1::dsred) and a dopaminergic marker (dat-1::mcherry). Our exhaustive TF RNAi screen has allowed us to identify 94 TFs candidates to be involved in the development of the different monoaminergic neurons. In this work we will present an overview of our screen as well as the additional characterization we are performing with some of these candidate genes.

P-22 Imidacloprid-containing pesticides disrupt C. elegans development

Beatrix Bradford, Paula Checchi
Marist College

Neonicotinoids are a class of pesticide that has been under investigation by the Environmental Protection Agency (EPA) in recent years. A major concern is a possible role they may play in colony collapse disorder in honey bees, wherein neonicotinoids disrupt both their development and behavior. One example of a neonicotinoid is imidacloprid, an active ingredient in pesticides. Here, we investigated the effects of the imidacloprid-containing pesticide Bayer Tree and ShrubTM on the development of the C. elegans germ line, a population of cells that is of particular importance as the exposure to toxins can affect subsequent generations. To determine the effects of imidicloprid on germ cell development, we scored apoptotic nuclei and found that imidacloprid caused a statistically significant increase in the number of corpses as a result of Bayer Tree and ShrubTM exposure. Accordingly, we also discovered a reduction in fertility in animals exposed to Bayer Tree and Shrub as determined by brood size analysis. We are currently exploring the nature of these defects, which strongly indicate that imidacloprid negatively impacts development and reduces gamete quality. Our data support EPA findings that neonicotinoids cause widespread harm to multiple species and raise concern about their safety to humans.

P-24 Some maternal mRNA transcripts are not homogenously distributed in early C. elegans embryos

Dylan M Parker, Marc T Nishimura, Sam Boyson, Erin Osborne Nishimura

Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO, USA

Previously, we performed single-cell RNA-seq on individual blastomeres from Caenorhabditis elegans early embryos. We identified hundreds of transcripts with cell-specific distribution prior to the onset of zygotic transcription (PMID: 25875092; PMID: 27554860). We aim to identify mechanisms driving cell-specificity of these mRNA transcripts. Microscopic imagining (smFISH) of a subset of these transcripts not only verified their cell-specific distribution, but surprisingly revealed distinct sub-cellular localization patterns, as well. Namely, the AB-enriched erm-1 (ezrin/radixin/moesin) and imb-2 (importin beta family) mRNAs shared the same localization as the proteins they encode: cell peripheral and nuclear peripheral, respectively. In contrast, P1-enriched transcripts chs-1 (chitin synthase), cpg-2 (chondroitin proteoglycan), and clu-1 (yeast mitochondrial clustering related) clustered together as did nos-2 (nanos related), a transcript previously shown to co-localize with P granules (PMID: 10518502). The 3'UTRs of erm-1 and imb-2 were insufficient to localize GFP mRNA to sub-cellular locales. In contrast, the nos-2 3'UTR was sufficient to localize GFP mRNA into granules. Further, we tested whether P-enriched, granularly-clustered mRNA transcripts co-localized with P granule marker proteins. Though chs-1and clu-1 did overlap with P granules they also appeared in clusters distinct of P granule proteins. In contrast, nos-2 and cpg-2 mRNA transcripts more exclusively overlapped with P granule protein markers. Together, these results underscore potential heterogeneity within RNP granules. We continue to test for mRNA sequences, RNA binding proteins, and RNA decay pathways required for cell-specific and sub-cellular mRNA patterning. In addition, we are examining whether signal peptides and translation, in general, play a role in directing mRNA transcripts into interesting sub-cellular distribution patterns.

P-26 EMR-1/emerin is involved in tissue-specific anchoring of chromatin to the nuclear envelope and neuromuscular junction activity

Celia M. Muñoz Jiménez, Cristina Ayuso, Agnieszka Dobrzynska, Peter Askjaer

CABD/CSIC/UPO

The nuclear envelope (NE) regulates transport of macromolecules between the nucleus and the cytoplasm and plays critical roles in nuclear organization and gene expression. Specific chromatin domains are anchored to the NE, which generally is associated with formation of heterochromatin and transcriptionally silencing. Reflecting the importance of NE functions, a broad range of human diseases are attributed to alterations in NE structure. Most notably are the laminopathies, with one example being Emery-Dreifuss muscular dystrophy (EDMD), which is caused by mutations in the inner nuclear membrane protein emerin or in the nuclear lamina protein lamin A. We recently found that EMR-1, the Caenorhabditis elegans ortholog of emerin, associates with genes implicated in muscle and nervous system function and regulates their expression. Interestingly, deletion of emr-1 causes local changes in nuclear architecture and hypersensitivity to the cholinesterase inhibitor aldicarb, indicating altered activity at neuromuscular junctions (NMJ). Although many NE proteins are ubiquitously expressed, laminopathies often affect a single tissue. It is hypothesized that tissue-specific alterations in nuclear organization might be responsible for particular symptoms of laminopathies. We suggested that emerin might regulate nuclear organization in a tissue-specific manner. For this reason, we developed a novel FLP-Frt-based DamID technique to study changes in interactions between EMR-1 and chromatin in specific cell types using intact animals as starting material. Interestingly, EMR-1 interaction profiles in muscles vs. neurons reveal both many common domains but also local changes in chromatin organization. Generally, most gene classes are more frequently in contact with EMR-1 in neurons as compared to muscles. Within the two tissues, silent genes are more associated with EMR-1 than active genes, although we also observed exceptions where EMR-1 was enriched at active genes. This suggests that EMR-1 might have both repressive and activating roles at the NE and we are testing how this influence NMJ activity.

P-28 Longevity and its transgenerational inheritance is enabled by repressive chromatin

Teresa Lee, Amanda Engstrom, David Katz

Department of Cell Biology, Emory University, Atlanta GA, USA

Longevity is a complex trait influenced by a mix of environmental, genetic, and epigenetic factors. WDR-5, a member of the COMPASS complex, methylates histone 3 at lysine 4 (H3K4), a modification associated with active chromatin. Previously, the Brunet lab has shown that wdr-5 mutants are long-lived, and this longevity is inherited by wild-type descendants. We demonstrate that longevity in this background is a transgenerational phenotype that takes at least 18 generations to manifest after the loss of WDR-5. Consistent with the gradual appearance of longevity in wdr-5 mutant populations, we see that lifespan correlates with levels of dimethylation of histone 3 at lysine 9 (H3K9me2), a mark associated with repressive chromatin. Using ChIP-seq to examine global levels of H3K9me2 in wdr-5 mutants, we find that late-generation, long-lived wdr-5 mutants have higher levels of genomic H3K9me2 both globally and at locus-specific regions. To examine whether H3K9me2 may confer longevity in wdr-5 mutants through its inheritance and generational accumulation, we mutated met-2, the methyltransferase required for all germline H3K9me2, in wdr-5 mutants. The extended lifespan of wdr-5 mutants is dependent on met-2, indicating that H3K9me2 promotes longevity in late-generation wdr-5 mutants. Additionally, when we mutated met-2 in otherwise wild-type descendants of wdr-5 mutants, we find that its loss can also abolish the inheritance of longevity. Taken together, these data support a model in which germline H3K9me2 is transgenerationally inherited, thereby facilitating longevity and its epigenetic inheritance.

P-30 The subapical intermediate filament-rich endotube responds to and protects against microbial insults and toxins in the C. elegans intestine

Florian Geisler[1], Richard A. Coch[1], Christine Richardson[2], Martin Goldberg[2], Olaf Bossinger[3], Rudolf E. Leube[1]
[1]Institute of Molecular and Cellular Anatomy, RWTH Aachen University, Aachen, Germany
[2]School of Biological and Biomedical Sciences, Durham University, Durham, United Kingdom
[3]Institute of Anatomy I, Molecular Cell Biology, University of Cologne, Cologne, Germany

The lumen of the C. elegans intestine is circumferentially surrounded by a dense sheath of intermediate filaments that is attached to the C. elegans apical junction and is referred to as the endotube. It is evolutionarily conserved corresponding to the desmosome-anchored intermediate filament-rich layer in the intestine of vertebrates. To test its role in responding to microbial infections and toxins, we studied the reaction of the endotube to nematicidal Bacillus thuringiensis strain DB27 and the pore-forming toxin Cry5B. We find drastic alterations in endotube structure and compensatory changes in intermediate filament protein expression upon infection with the microbe or treatment with Cry5B. To elucidate whether the endotube acts as a protective barrier, we examined the toxin response of strains with a locally perturbed endotube (gene sma-5(n678)), complete endotube loss (gene ifb-2(kc14)) and endotube loss with coincident intermediate filament aggregates at the C. elegans apical junction and in the cytoplasm (gene ifo-1(kc2)). We observe that the mutant strains show drastically increased larval arrest, prolonged time of larval development and reduced survival. The severity of the phenotype reflects the severity of endotube alterations noted in the different mutant strains and correlates with reduced rescue of larval arrest upon toxin removal. Our results can be taken as in vivo evidence for a major protective role of intermediate filaments composing an additional intracellular barrier in the subapical domain of intestinal cells. The findings are of relevance across the animal kingdom all the way to humans given the evolutionary conservation of the polarized distribution of the intestinal intermediate filament system.

P-32 Identification of protein-protein interactions at the nuclear envelope

Agnieszka Dobrzynska, Javier Macías-León, Triana Solís-Vázquez, Carmen Espejo Serrano, Cristina Ayuso, Peter Askjaer
Andalusian Centre for Developmental Biology, CSIC/Universidad Pablo de Olavide/Junta de Andalucia, Seville, Spain

The nuclear envelope (NE) is involved in numerous biological processes, including cellular compartmentalization, control of nucleocytoplasmic transport, genome organization, DNA replication, repair and transcription. Reflecting the diversity and complexity of these processes, several hundreds proteins are reported to accumulate at the NE. Interactions between NE components are incompletely mapped, which hinders full understanding of NE biogenesis and functions. Phosphorylation of NE proteins is critical for NE breakdown at entry to mitosis. We previously

described that VRK-1 (Vaccinia-Related Kinase 1) phosphorylates BAF-1. BAF-1 anchors chromatin to the NE via nuclear lamins and inner nuclear membrane proteins, such as EMR-1/emerin and LEM-2. In the absence of VRK-1, BAF-1 remains chromosome-bound upon mitotic entry and defects in chromosome segregation are observed. VRK-1 is also expressed in postmitotic cells and to identify novel partners we immunoprecipitated VRK1 from human cells followed by mass spectrometry analysis. The top candidates include several NE and chromatin-associated proteins, including BAF and emerin, transcription factors and chromodomain proteins. We are applying a variety of methods, such as yeast two-hybrid assays and surface plasmon resonance technology to verify these interactions. In addition, we are employing systems to study protein-protein interactions in vivo by protein fragment complementation assays based on BiFC and NanoLuc technologies. These systems enable us to test VRK-1 interactions at physiological expression levels and subcellular resolution, but they can be adapted to any other protein pair. In conclusion, we have validated several novel VRK-1 interaction partners, broadening the implication of VRK-1 in chromatin processes.

P-38 piChIP: a single locus IP technology to identify novel factors in the C. elegans germline Nuclear RNAi pathway

Ahmet Can Berkyurek[1, 2], Guilia Furlan[1, 2], Alper Akay[1, 2], Fabian Braukmann[1, 2], Peter Dimaggio[4], Eric Miska[1, 2, 3]

[1]*The Gurdon Institute, University of Cambridge, UK*

[2]*The Department of Genetics, University of Cambridge, UK*

[3]*Sanger Institute, Cambridge, UK*

[4]*Department of Chemical Engineering, Imperial College, London, UK*

Non-self DNA such as transposable elements have the ability to copy and move themselves within the host genome and hence representing a threat to the genome stability and integrity. To prevent the spreading of these selfish elements, organisms have developed defence mechanisms dependent on small RNA pathways, with the piRNAs being one of the silencing machineries in the nematode C. elegans germline. Apart from the basic understanding of chromatin regulation mediated by the histone H3K9me3, H3K27me3 marks and specific chromatin binding proteins, little is known about the spatial and temporal regulation of chromatin in the piRNA pathway. Toward this goal, we have developed a germline specific single locus ChIP system (piChIP) by generating a transgenic lines harbouring a piRNA activity reporter to identify proteins, small and long RNAs interacting with a single piRNA target locus. piChIP-RNAseq clearly recovers anti-sense 22G RNAs mapping to the exons of the reporter nascent transcript, reflecting the ability of our piChIP system to specifically pull down small RNAs targeted to a specific piRNA target locus during co-transcriptional silencing. Next, we plan to characterize the proteins associated with a single piRNA locus by TMT proteomics.

P-40 Modeling human RASopathies in vulval development to dissect the molecular mechanisms and identify novel disease-genes

Luca Pannone[1, 2], Simona Coppola[3], Emanuela Pone[4], Francesca Pantaleoni[1], Ivan Gallotta[4], Elia Di Schiavi[4], Marco Tartaglia[1], Simone Martinelli[2]

[1]*Genetics and Rare Diseases Research Division, Ospedale Pediatrico Bambino Gesù, IRCCS, Rome, Italy*

[2]*Department of Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome, Italy*

[3]*National Center for Rare Diseases, Istituto Superiore di Sanità, Rome, Italy*

[4]*Institute of Biosciences and Bioresources, National Research Council, Naples, Italy*

RASopathies are a family of clinically related disorders affecting development and growth. Past work allowed us to identify the invariant p.Ser2Gly substitution affecting SHOC2 and the Gly39 duplication in RRAS as molecular events underlying two different RASopathies. Ubiquitous expression of these mutants in C. elegans was shown to alter vulval development, eliciting protruding vulva (Pvl). Here, we used the nematode to dissect the impact of SHOC2 and RRAS mutations on signal transduction and highlight novel candidate disease-genes. Genetic analyses established that SUR-8/SHOC2 and RAS-1/RRAS work in the same pathway to induce Pvl. Both RAS-1 and RAS-2/MRAS are downstream to constitutively active SHOC2, the former being epistatic to the latter. Specific

overexpression of SHOC2S2G or RRASG39dup in vulval precursor cells engendered Pvl and multiple ectopic pseudovulvae (Muv), a phenotype associated with upregulation of LET-60/RAS. RNA interference experiments confirmed the involvement of LET-60 in promoting Muv, excluded a role for AGE-1/p110 (PI3Kc) and RHO-1/RHOA in vulval defects, and established that MIG-2, CED-10/RAC1 hyperactivation underlies Pvl in a LET-60-independent manner. A counteracting effect on this phenotype was exerted by CDC-42. C. elegans data were validated in humans by showing that SHOC2S2G causes constitutive RAC1 activation and cytoskeletal alterations in transfected cell lines and patient's-derived fibroblasts. These findings suggest the contribution of dysregulated signaling controlling cell migration to certain features of RASopathies (e.g., lymphedema, cardiac defects, JMML) and highlight novel candidate disease genes for these mendelian traits. In line with this evidence, we have recently reported that missense mutations in CDC42 underlie a clinically heterogeneous group of diseases characterized by growth dysregulation, facial dysmorphism, and neurodevelopmental, immunological and hematological anomalies, including a phenotype resembling Noonan syndrome, the most common RASopathy (Martinelli et al., 2018. Am J Hum Genet. doi: 10.1016/j.ajhg.2017.12.015).

P-42 Generation and validation of a microfluidic platform for high-throughput detection of cancer metabolites in urine samples

Martina Di Rocco[1, 2, 3], Enrico Lanza[1, 3], Davide Caprini[4], Luca Pannone[2, 5], Simone Martinelli[2], Giancarlo Ruocco[1, 6], Viola Folli[1]

[1]Center for Life Nanoscience, Istituto Italiano di Tecnologia, Rome, Italy

[2]Department of Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome, Italy

[3]Department of Cellular Biotechnology and Haematology, Sapienza, University of Rome, Rome, Italy

[4]Department of Mechanical and Aerospace Engineering (DIMA), Sapienza University of Rome, Rome, Italy

[5]Genetics and Rare Disease Research Division, Ospedale Pediatrico Bambino Gesù, IRCCS, Rome, Italy

[6]Department of Physics, Sapienza, University of Rome, Rome, Italy

The discovery of cheap and non-invasive biomarkers for early detection of cancer is an urgent priority. Taking advantage from the extraordinary sense of smell of dogs and mice, previous studies demonstrated the existence of cancer-specific odorants in biological fluids. More recently, it has been shown that C. elegans displays attractive chemotaxis towards urine from patients with tumors, but avoids those from healthy subjects. Aim of this work is to develop a microfluidic lab-on-chip platform for detection of cancer metabolites in urine from women with breast cancer (BC) by using chemotaxis assays and calcium imaging analysis. The former strategy was performed on 26 samples from BC patients and 23 from control individuals, and confirmed the ability of animals (N2, Bristol) to sense cancer metabolites, with a sensibility and a specificity of approximately 85%. Additional 25 samples per group are already available in the lab and will be tested within the next three months. The response was not significantly affected by cigarette smoking; rather, the chemotaxis index (CI) was strongly influenced by hormones in fertile women. Specifically, we observed a positive correlation between CI and LH, FSH and estradiol release preceding ovulation, and the progesterone peak during the luteal phase. In one cyclist, the positive CI was normalized after two weeks bike-free, suggesting an attractive behavior towards human PSA. To study the activation of individual neurons in animals with a timely controlled odor exposition, a microfluidic device was generated. Analyses were performed in animals expressing a genetically encoded calcium indicator (GCaMP) in the AWC-on sensory neuron. A clear fluorescence signal was evident when cancer urines were presented, while no/less activity was recorded with control samples. Although preliminary, our findings constitute a proof of concept for the development of a microfluidic device for early detection of cancer metabolites in urine.

P-44 A novel correction mechanism regulates nuclear position and ensures proper DNA segregation during late cytokinesis

Anne Pacquelet, Matthieu Jousseaume, Grégoire Michaux

CNRS, Institut de Génétique et de Développement de Rennes

Ensuring correct DNA segregation is an essential feature of cell division which relies on the proper assembly of the mitotic spindle and its coordination with the cytokinetic machinery. Here we present a novel mechanism which corrects DNA segregation defects due to cytokinetic furrow mispositioning. We previously showed that tight regulation of myosin is required to coordinate furrow and spindle positions during the first division of C. elegans embryos: abnormal accumulation of myosin at the anterior cortex induces a strong displacement of the furrow towards the anterior, thereby uncoupling cytokinetic furrow and spindle positions and leading to DNA segregation defects (Pacquelet et al;, J Cell Biol, 210,1085). However, we unexpectedly found that these DNA segregation defects can be corrected at the end of cytokinesis. This correction occurs when the mitotic spindle midzone is being disassembled and after nuclear envelop reformation. It relies on the concomitant displacement of the furrow and of the anterior nucleus towards the posterior and anterior poles, respectively. Those displacements coincide with an anteriorly directed flow of cytoplasmic particles. Genetic experiments demonstrated that the displacement of the anterior nucleus requires the interaction of the nucleus with microtubules as well as myosin activity. Moreover, both furrow displacement and cytoplasmic flow also require myosin activity. These results lead us to propose that myosin regulates nuclear position by inducing furrow displacement, which would in turn increase intracellular pressure in the posterior region of the embryo and create an anteriorly directed cytoplasmic flow. Altogether, our work reveals the existence of a so far undescribed correction mechanism which ensures that DNA segregation defects due to the mispositioning of the cytokinetic furrow are corrected during late cytokinesis. This correction involves the regulation of nuclear position by the concomitant action of microtubules and myosin and is critical to ensure the robustness of cell division.

P-46 Long-term monitoring of cytosolic and mitochondrial Ca2+ dynamics in C. elegans pharynx

Pilar Álvarez[1], Paloma Garcia-Casas[1], Jessica Arias-del-Val[1], Adolfo Sanchez-Blanco[2], Rosalba I Fonteriz[1], Javier Alvarez[1], Mayte Montero[1]

[1]Institute of Biology and Molecular Genetics (IBGM), Department of Biochemistry and Molecular Biology and Physiology, Faculty of Medicine, University of Valladolid and CSIC, Ramón y Cajal, 7, E-47005 Valladolid, SPAIN.

[2]Department of Biology, University of Hartford, West Hartford, Connecticut 06117, USA

We have expressed a Ca2+ sensor either in the cytosol or in the mitochondrial matrix of C. elegans pharynx cells and we have measured cytosolic and mitochondrial [Ca2+] dynamics in the pharynx of live C. elegans worms during aging. Despite the age-dependent decline of pharynx pumping, we observed unaltered, fast Ca2+ oscillations both in young and old worms. In addition, sporadic prolonged Ca2+ increases lasting many seconds or minutes were often observed in between periods of fast Ca2+ oscillations. Similar [Ca2+] transients were observed both in cytosol and in mitochondria, showing that mitochondrial [Ca2+] is able to follow “beat-to-beat” the fast oscillations of cytosolic [Ca2+]. Food deprivation largely augmented the frequency of prolonged [Ca2+] increases, suggesting that they are due to the inhibition of ATP-dependent Ca2+-pumps upon energy depletion. However, paradoxically, prolonged [Ca2+] increases were more frequently observed in young worms than in older ones, and less frequently observed in energy-deficient mitochondrial respiratory chain nuo-6 mutants than in wild-type controls. We hypothesize that young animals are more susceptible to energy depletion due to their faster energy consumption rate, while nuo-6 mutants may keep better the energy balance by slowing energy consumption. Regarding mitochondria, Ca2+ fluxes in and out of the mitochondria are relatively well preserved during the C. elegans life, but there is a clear progressive decrease in their magnitude during aging. Moreover, mitochondrial Ca2+ fluxes were smaller in nuo-6 mutants with respect to the controls at every age and decreased similarly during aging.

P-48 The identification and characterization of chromatin regulators involved in coelomocyte to neuron conversion in C. elegans

Ismail Özcan[1], Anna Reid[1], Margaux Quiniou[1, 2], Andreas Ofenbauer[1], Baris Tursun[1]

[1]The Berlin Institute of Medical Systems Biology (BIMSB) at the Max Delbrück Center for Molecular Medicine (MDC) in the Helmholtz Association, Berlin, Germany

[2]University of Exeter, Exeter, United Kingdom

Reprogramming differentiated somatic cells into neurons is one of the frontiers of current neuroscience research. We have developed a new system to understand the mechanisms of transcription factor (TF)-induced transdifferentiation (Td) to neurons based on the zinc finger TF CHE-1 that specifies the identity of gustatory ASE neurons in C. elegans (Tursun et al., Science, 2011). Using this system, we discovered that ectopic expression of the TF CHE-1 directly reprograms coelomocytes (CCs), which are mesodermal cells with scavenging and hepatic functions, into ASE neuron-like cells. Notably, differentiated CCs express an ASE neuron-specific reporter, show neuron-like cell morphogenesis such as neuron-like projections, and, in addition, express a pan-neuronal marker and stain for the synaptic protein UNC-10 (Rim1 homolog). Individual animals show varying degrees of CC to ASE neuron conversion with converted CCs displaying neuronal morphologies or partially converted CCs. Interestingly, a portion of the population, approximately 40%, demonstrate no CC to neuron-like cell conversion, indicating that inhibitory mechanisms or barriers are restricting Td. To uncover the mechanisms behind CC Td at the chromatin level, we performed a chromatin sub-library RNAi screen. We have identified several candidates that, when knocked down, enhance or suppress conversion and may, therefore, play an important role during Td. Among our candidate barrier factors, we have observed an increase in conversion efficiency from 60% up to 82%. In addition, functional enrichment analysis indicates that our putative enhancers and suppressors show molecular interactions. By exploring the role of our candidate enhancers and suppressors in Td we aim to tease apart the mechanisms by which they function, which may contribute to our understanding of how neurons can be generated by Td from other cells and tissues.

P-50 Importance of the glutamate synthase homologue (W07E11.1) for lifespan extension and stress resistance in the C. elegans daf-2 mutant

Aleksandra Zečić, Bart Braeckman

Biology Department, Ghent University, 9000 Ghent, Belgium

Reduction of insulin/insulin-like growth factor signalling (IIS) due to daf-2 mutation doubles the lifespan of adult C. elegans through the nuclear translocation and activation of the FOXO transcription factor DAF-16. Furthermore, in C. elegans, reduced IIS results in extensively restructured metabolism and increased resistance to various types of stress. Our previous proteomic study of the daf-2(e1370) mutant has identified upregulation of many enzymes involved in carbohydrate, lipid and amino acid metabolism. One of the top upregulated proteins (16.6x) is a putative glutamate synthase (W07E11.1). Currently, there is no functional information available on this C. elegans gene, which is absent in vertebrates. We hypothesised that due to a drastic reduction in protein synthesis in daf-2 worms most amino acids are catabolised, resulting in a large glutamate pool. This excess glutamate might be converted into glutamine and α-ketoglutarate via the upregulated glutamate synthase, which may be the key to alternative carbon and nitrogen flows that are observed in daf-2 mutants: it may redirect amino acid carbons to the TCA cycle, while nitrogen may be redirected to purine and uric acid synthesis for temporary storage. Given the previously shown importance of carbon-rich compounds (e.g. glycogen and trehalose) for daf-2 longevity and stress resistance, we expected that lack of glutamate synthase will impair these phenotypes in daf-2 worms. To test this, we performed the RNAi knockdown of the glutamate synthase homologue in daf-2 mutants and wild-type worms and measured its impact on their longevity and resistance to heat, oxidative, osmotic and UV stress. Our findings indicate that glutamate synthase has no effect on daf-2 lifespan extension, heat, oxidative and osmotic stress. However, the knock down of this enzyme heavily impairs daf-2 survival under UV stress, which supports previous studies that uncoupled stress resistance from longevity phenotypes in longevity mutants.

P-52 Loss of glutathione redox homeostasis impairs proteostasis by collapse of autophagy-dependent protein degradation

David Guerrero-Gómez[1], José Antonio Mora-Lorca[1], Beatriz Sáenz-Narciso[2], Francisco José Naranjo-Galindo[1], Fernando Muñoz-Lobato[1], Christopher D. Link[3], Christian Neri[4], Rafael Vázquez-Manrique[5], Peter Askjaer[6], Juan Cabello[2], Antonio Miranda-Vizuete[1]

[1]*Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, 41013 Sevilla, Spain*
[2]*Center for Biomedical Research of La Rioja, 26006 Logroño, Spain*
[3]*Department of Integrative Physiology, Institute for Behavioral Genetics, University of Colorado at Boulder, Boulder, CO, 80309 USA*
[4]*Sorbonnes Université, Centre National de la Recherche Scientifique, Research Unit Biology of Adaptation and Aging (B2A), Team Compensation in Neurodegenerative and Aging (Brain-C), F-75252, Paris, France*
[5]*Research Group in Molecular, Cellular and Genomic Biomedicine, Health Research, Institute-La Fe, 46026 Valencia, Spain; CIBER de Enfermedades Raras (CIBERER), Valencia, Spain.*
[6]*Andalusian Center for Developmental Biology (CABD), CSIC/JA/Universidad Pablo de Olavide, 41013 Seville, Spain*
Under non-stressed conditions, the redox status of the different subcellular compartments is tightly controlled. Hence, the cytoplasm has a reducing environment that favours cysteine protein residues in their dithiol form while the endoplasmic reticulum environment is oxidizing to facilitate the formation of disulfide bonds for protein folding. This situation is reversed in the presence of aggregation-prone proteins, as both compartments undergo a dramatic shift in their respective redox status, with the cytoplasm becoming more oxidized and the endoplasmic reticulum more reducing. However, whether changes in the cellular redox status affect protein aggregation has not yet been addressed. We show here that C. elegans mutants lacking glutathione reductase gsr-1, the enzyme responsible for recycling oxidized glutathione (GSSG) and thus maintenance of glutathione redox homeostasis, enhance the deleterious phenotypes of worm disease models caused by aggregating proteins like human b-amyloid peptide, a-synuclein or polyglutamine repeats containing proteins. Importantly, gsr-1 dependent proteostatic disruption is also found in C. elegans strains expressing endogenous UNC-52 and LET-60 aggregate-prone metastable proteins. This deleterious effect is largely phenocopied by the GSH depleting agent diethyl maleate. Protein aggregates can be disposed by autophagy and consistent with a role of GSR-1 in this process, gsr-1 mutants abolish nuclear translocation of the TFEB/HLH-30 transcription factor (a key mediator of autophagy induction) and strongly impair the degradation of the autophagy substrate p62/SQST-1::GFP. In agreement, genetic disruption of autophagy in gsr-1 mutants expressing aggregation prone proteins resulted in strong synthetic developmental phenotypes and in some cases lethality. Downregulation of glutathione reductase and GSH levels in both yeast and mammalian cell models also cause phenotypes associated to protein aggregation. Together, this study demonstrates a novel, evolutionarily conserved role of glutathione redox homeostasis in proteostasis maintenance through autophagy regulation.

P-54 Identification of potent drug candidates for attenuation of Cisplatin-induced neurotoxicity in the model organism C. elegans

Anna Wellenberg[1], Lea Weides[1], Julia Bornhorst[3], Barbara Crone[2], Uwe Karst[2], Gerhard Fritz[1], Sebastian Honnen[1]
[1]*Institute of Toxicology, Medical Faculty, Heinrich Heine University, Universitätsstraße 1, D-40225 Düsseldorf*
[2]*Institute of Inorganic and Analytical Chemistry, University of Muenster, Corrensstraße 30, D-48149 Muenster*
[3]*Institute of Nutritional Science, University of Potsdam, Arthur-Scheunert-Allee 114-116, D-14558 Nuthetal*
Cancer is the second leading cause of death worldwide and anticancer therapies are accompanied by severe adverse effects. One of the most common dose-limiting side-effects in using cis- or oxaliplatin is chemotherapy induced peripheral neuropathy (CIPN). Its pathophysiology is unknown and neither prophylaxis nor specific treatment is available. Therefore, further research elucidating the underlying molecular mechanisms of CIPN caused by platinating anti-tumor drugs is required as basis for future development of preventive or therapeutic strategies. Here, we used C. elegans as an alternative test system to converge underlying mechanisms of platinum-induced neurotoxicity. We proved dose dependent uptake of cisplatin by C. elegans using ICP-MS. Correspondingly we detected also a dose dependent induction of typical platinum-derived DNA 1,2-GpG-intrastrand crosslinks by south-western blotting for cis- and carboplatin, which were repaired within 24 h recovery time. The tested concentrations were sufficient to induce apoptosis in C. elegans. Interestingly we found that doses with only little effect on development, reproduction and musculature led to strong (cis- and oxaliplatin) neurotoxicity as demonstrated by impaired pharyngeal pumping and chemotaxis. Exposure to platinating agents sensitized C. elegans to oxidative

stress (H2O2, paraquat). This seems to be caused by glutathione (GSH) depletion without any increase of glutathione disulfide (GSSG) or general ROS-levels. In fact, a decline of endogenous GSH production via RNAi knockdown of the Glutamate Cysteine Ligase was sufficient to induce neuropathic effects comparable to cisplatin. Consequently the reduction in oxidative stress resistance could be prevented by N-acetylcysteine (NAC). Recently we observed that NAC, acetylsalicylic acid and WR1065 (the active metabolite of Amifostine) reduce neurotoxic effects of platinum compounds in a post-treatment scheme. Interestingly, the amount of platinum determined in such nematode populations was largely unchanged in comparison to the control groups. The apoptosis induction was not influenced by these treatments.

P-56 A natural molecular variant enhancing C. elegans dauer formation in response to diverse environmental cues

Bénédicte Billard[1, 3], Paul Vigne[1, 3, 4], Clotilde Gimond[1, 2, 3], Christian Braendle[1, 2, 3]

[1]*Institut de Biologie Valrose Nice*

[2]*CNRS*

[3]*University of Nice Sophia Antipolis*

[4]*Inserm*

Virtually all organisms possess the capacity to flexibly adjust their development in response to environmental changes. A prime example of such adaptive developmental plasticity is dauer formation in the nematode C. elegans whereby larvae can adopt an alternative, stress-resistant larval stage (termed dauer) in response harsh environmental conditions (e.g. high population density, starvation, or high temperature). The molecular mechanisms regulating dauer induction have been well-characterized and involve Insulin, TGF-Beta and steroid signalling. In contrast, few studies have determined the molecular variants explaining differences in dauer induction among natural C. elegans isolates. Here we characterized a C. elegans isolate (JU751, France), which shows an unusually strong propensity to form dauers at relatively low population densities compared to most other C. elegans isolates. Therefore, we performed a QTL analysis using F2 recombinant inbred lines (RILs) generated from an intercross between JU751 and another isolate (JU1200, UK) displaying dauer induction only under high density and other harsh environmental conditions. This analysis identified a single highly significant QTL on chromosome III, spanning approximately 700kb. We then confirmed the effect of this QTL region on dauer induction through establishment of near-isogenic lines (NILs). After further restricting the target region through fine-mapping, we focused on a single candidate variant, a 90bp deletion in the presumptive promoter region of eak-3, a gene known to be involved in dauer induction in response to high temperature. We present experimental evidence that this gene regulatory change is indeed the causal basis for the evolution of increased dauer induction in the JU751 isolate – in response to multiple, diverse environmental cues. Our result connects with classical developmental genetic studies to allow for precise identification of evolutionary changes in well-characterized signalling networks regulating adaptive developmental plasticity.

P-58 Insulin-dependent quiescence and arrest at hatching

Bruce Wightman

Muhlenberg College, Allentown, PA 18104 USA

The fax-1 nuclear hormone receptor and unc-42 homeobox gene control interneuron identities in C. elegans. The fax-1 and unc-42 transcription factors function in specifying the identities of an overlapping subset of nematode interneurons, including the command interneurons AVA and AVE, which function in coordinated movements. Both genes are required for the expression of neuron-specific genes, including glutamate receptor subunits, as well as axon pathfinding and other aspects of interneuron identity. Both fax-1 and unc-42 mutations cause a fully-penetrant peri-hatching arrest in combination with a weak daf-2 insulin receptor mutation. The arrest can be reversed by a mutation in the daf-16 forkhead transcription factor, which functions downstream of daf-2, but not by mutations in the parallel TGFb pathway. Daf-c mutations in the TGFb and steroid pathways do not cause a synthetic arrest phenotype in combination with either fax-1 or unc-42 mutations. Arrested fax-1;daf-2 and unc-42;daf-2 embryos

typically display normal L1 morphology, but often remain coiled in a broken eggshell in a state of quiescence with weak or absent pharyngeal pumping. Arrested embryos can be prompted to vigorous movement by stimulation with blue light. The arrest phenotype can also be partially reversed by a mutation in egl-4, which is required for sleep-like quiescence. Temperature-shift experiments suggest that embryogenesis is the critical stage for quiescence and arrest. These observations indicate that the fax-1 and unc-42 transcription factors function in an insulin pathway, or a convergent parallel pathway, that controls arousal and developmental progression out of embryogenesis. Given that both fax-1 and unc-42 are required for the development of a limited set of interneurons, these experiments suggest a previously unappreciated role for interneuron function and insulin signaling in regulating developmental arrest and arousal. Supported by NIGMS.

P-60 A link between C.elegans morphogenesis and mRNA export.

Angelina Zheleva[1], Eva Gomez-Orte[1], Beatriz Saenz-Narciso[1], Begoña Ezcurra[1], Maria de Toro[1], Henok Kassahun[2], Hilde Nilsen[2], Ralf Schnabel[3], Juan Cabello[1]

[1]CIBIR (Center for Biomedical Research of La Rioja), Piqueras 98. Logroño. 26006 La Rioja. Spain.

[2]Institute of Clinical Medicine, Department of Clinical Molecular Biology, University of Oslo and Akershus University Hospital, 1478 Lørenskog, Norway.

[3]Department of Developmental Genetics, Institute of Genetics, Technische Universität Braunschweig, 38106, Germany.

Multiple studies have explored the mechanisms governing development of the Caenorhabditis elegans pharynx. In this study we used whole genome sequencing (WGS) and CloudMap/Hawaiian Variant Mapping to specifically map a mutation that produced a Pun pharynx (unattached pharynx) phenotype. We discovered a thermo-sensitive t2160 mutant allele of nuclear export 1, nxf-1. nxf-1/TAP encodes a protein that is required for mRNA export from the nucleus to the cytoplasm. We visualised pharyngeal morphogenesis using membrane and nuclear PHA-4::GFP reporters. Our analysis suggests that nxf-1 plays a role in pharynx attachment by affecting arcade cell-cell membrane contacts and the actin filament network in the arcade cells. To better understand the role of NXF-1 in C. elegans embryonic morphogenesis, we analysed the expression of several apical junction markers in nxf-1(t2160) homozygous embryos and we showed that nxf-1(t2160) arcade cells failed to generate apical junctions. Additionally, depletion of the mRNA export machinery, NXF-1, NXT-1 and HEL-1, leads to embryonic lethality and Pun phenotype. We suggest that some tissues are more sensitive during the developmental process, especially epithelial tissues such as the C. elegans pharynx and epidermis, which seem to need tight regulation of mRNA export. In addition, our transcriptomic analysis reveals a probable feedback loop by which the mutation of nxf-1(t2160) affects the export of mRNA, causing the overexpression of genes involved in this process and mRNA surveillance pathway. Our results suggests a model of RNA export and recycling of transporter proteins back to the nucleus from the cytoplasm.

P-62 Effect of the diet type and temperature on the C. elegans transcriptome

Eva Gómez-Orte[1], Eric Cornes[2], Angelina Zheleva[1], Beatriz Saenz-Narciso[1], Maria de Toro[1], Maria Iniguez[1], Rosario Lopez[1], Begoña Ezcurra[1], Adolfo Sanchez-Blanco[3], Julian Ceron[2], Juan Cabello[1]

[1]CIBIR (Center for Biomedical Research of La Rioja), Piqueras 98, Logroño, 26006 La Rioja, Spain

[2]Bellvitge Biomedical Research Institute - IDIBELL, L'Hospitalet de Llobregat, 08908 Barcelona, Spain

[3]Department of Biology, University of Hartford. 200 Bloomfield Ave, West Hartford, CT 06117, USA

The standard protocol for Caenorhabditis elegans growth and maintenance is 20ºC on an Escherichia coli diet. Temperatures ranging from 15ºC to 25ºC or feeding with other species of bacteria are considered physiological conditions, but the effect of these conditions on the worm transcriptome has not been well characterized. We compare the global gene expression profile for the reference Caenorhabditis elegans strain (N2) grown at 15oC, 20oC, and 25oC on two different diets, Escherichia coli and Bacillus subtilis. When C. elegans were fed E. coli and the growth temperature was increased, we observed an enhancement of defense response pathways and down-regulation of genes associated with metabolic functions. However, when C. elegans were fed B. subtilis and the growth temperature was increased, the nematodes exhibited a decrease in defense response pathways and an enhancement of expression of genes associated with metabolic functions. Our results show that C. elegans undergo

significant metabolic and defense response changes when the maintenance temperature fluctuates within the physiological range and that the degree of pathogenicity of the bacterial diet can further alter the worm transcriptome. We plan to extend these studies throughout the aging.

P-64 Actomyosin contractility regulators stabilize the cytoplasmic bridge of the primordial germ cells Z2 and Z3 during C. elegans embryogenesis.

Eugénie Goupil[1], Rana Amini[1], David H. Hall[3], Jean-Claude Labbé[1, 2]

[1]Institute of Research in Immunology and Cancer, Université de Montréal, Montréal, QC H3C 3J7, Canada.

[2]Department of Pathology and Cell Biology, Université de Montréal, Montréal, QC H3C 3J7, Canada.

[3]Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Cytokinesis, the physical separation of the daughter cells after mitosis, does not complete during germline development in most animals, leading to the formation of a stable cytoplasmic bridge and, eventually, a syncytium. Whereas the C. elegans adult germline is syncytial, the regulators implicated in its formation remain unknown. Using live imaging and electron microscopy, we found that the germline precursor blastomere, P4, fails cytokinesis, leaving a stable cytoplasmic bridge enriched in remnants of the contractile ring between the two primordial germ cells, Z2 and Z3. Depletion of several regulators of actomyosin contractility enabled proper initiation of P4 cytokinesis but resulted in a regression of the membrane partition between Z2 and Z3, later during embryogenesis, indicating their requirement to stabilize the cytoplasmic bridge during embryonic development. Epistatic analysis, using immunofluorescence, revealed a pathway in which Rho regulators promote the accumulation of the non-cannonical anillin ANI-2 at the stable cytoplasmic bridge. This enables the accumulation of the non-muscle myosin II NMY-2 and the midbody component CYK-7 at the bridge, in part by limiting the accumulation of canonical anillin ANI-1. Our results uncover key steps in C. elegans germline formation and define a set of conserved regulators that are enriched at the primordial germ cells cytoplasmic bridge to ensure its stability during embryonic development.

P-66 spe-51 (as39) is dispensable for early spermatogenesis and spermiogenesis but required for sperm function in C. elegans

Xue Mei, Gunasekaran Singaravelu, Marina Druzhinina, Sunny Dharia, Andrew Singson

Waksman Institute, Rutgers University

Fertilization occurs when the sperm and egg recognize and bind with each other and fuse to form a zygote. The molecular basis of sperm and egg interactions are largely unknown. In a forward genetic screen, we isolated a spermatogenesis-defective (spe) mutant, in which fertilization fails. Mutant hermaphrodites produce unshelled eggs with endomitotic nuclei, suggesting that the sperm and egg do not fuse. Mutant hermaphrodites produce progeny when mated with wild type males; while mutant males fail to sire outcross progeny. These observations suggest that the mutation only affect the male. Dissection of the males shows that they produce normal-looking spermatids. These spermatids can activate in vitro with pronase just as control spermatids do. We conclude that as39 mutation does not affect early spermatogenesis or spermiogenesis, but affects sperm function. as39 phenocopies the spe-9 class of sperm-function mutants. So far, all the identified spe-9 class of genes encodes trans-membrane proteins on the sperm surface that are thought to mediate the binding and/or adhesion of the sperm to the egg. Using whole-genome sequencing, we identified a nonsense mutation in the gene T22B11.1 in as39. Injection of genomic DNA of T22B11.1 rescues the sterility phenotype in as39. From here, we name T22B11.1 spe-51. This gene encodes a protein that is also predicted to be a trans-membrane molecule. In summary, we have identified the spe-51 gene that is dispensable for early spermatogenesis and spermiogenesis, but required for sperm function. Ongoing studies will focus on the localization of the SPE-51 protein and its interactions with other sperm-surface proteins.

P-68 Deciphering the role of centrosomes in breaking symmetry of the C. elegans zygote

Kerstin Klinkert, Coralie Busso, Sarah Herrman, Lukas von Tobel, Pierre Gönczy

Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, Swiss Federal Institute of Technology Lausanne (EPFL), Switzerland

The newly fertilized C. elegans zygote initially exhibits uniform RHO-1-dependent contractions of the acto-myosin cortex. Thereafter, symmetry of the system is broken, as evidenced by clearance of the RHO-1 guanine-nucleotide-exchange factor (GEF) ECT-2 from the cortex next to centrosomes, resulting in local cortical relaxation. This is followed by anterior-directed flows of the acto-myosin cortex and segregation of anterior and posterior PAR proteins into distinct cortical domains, with PAR-6 on the anterior and PAR-2 on the posterior. It has been shown that laser-mediated removal of centrosomes prevents polarity establishment (1), but the mechanisms through which centrosomes instruct symmetry breaking remain unclear. Intriguingly, we observed that zygotes from a mutant that lacks centrosomes undergo spontaneous symmetry breaking, leading to the formation of a PAR-2 domain either on the anterior, on the posterior or, most often, simultaneously on both poles. We found that such a bipolar phenotype is accompanied by weak cortical flows from both poles towards the center of the embryo. Likewise, preventing centrosome maturation by depleting SPD-2, SPD-5 or the Aurora A kinase AIR-1 leads to spontaneous symmetry breaking and a bipolar phenotype. Furthermore, we demonstrate that AIR-1 kinase activity is essential for proper symmetry breaking. By contrast, depletion of centrosomal components acting downstream of AIR-1 do not exhibit polarity defects, suggesting that AIR-1, in addition to centrosome maturation, is also critical for symmetry breaking. Overall, we demonstrate that centrosomes are required for the spatial regulation of symmetry breaking in an AIR-1-dependent manner. Furthermore, our findings reveal that C. elegans zygotes possess an unexpected intrinsic ability to break symmetry in a bipolar fashion in the absence of centrosomes. References: (1) Cowan CR, Hyman AA; Nature (2004)

P-70 Splicing-related Retinitis Pigmentosa mutations in C. elegans

Dmytro Kukhtar, Karinna Rubio-Peña, Xènia Serrat, Julián Cerón

Modelling human diseases in C. elegans Group. Bellvitge Biomedical Research Institute – IDIBELL Gran via 199, Hospitalet de Llobregat 08908, Barcelona, Spain

CRISPR enables fast genome editing to reproduce disease-causing mutations in model organisms. This approach allows us to study the impact of such mutations in the functionality of the gene. Introducing human mutations in invertebrates should be particularly informative for splicing-related diseases since the functions of splicing factors are very well conserved from yeast to mammals. In a previous study (Rubio-Peña et al 2015) we used RNAi to establish a model for splicing-related Retinitis Pigmentosa (s-RP) in C. elegans. s-RP genes are essential and most s-RP mutations are missense of aminoacids that are conserved from nematodes to mammals. In this context, we have reproduced s-RP mutations for the splicing factor genes prp-8/PRPF8 and snrp-200/SNRNP200/BRR2 in the C. elegans genome. By studying the penetrance of the phenotypes we can infer the clinical impact of these mutations. In other words, C. elegans can be used as a prognostic tool and a step forward in personalized medicine. Our s-RP model would have additional utilities: (i) screen for small molecules that alleviate the phenotypes, (ii) screen for genetic interactions with s-RP mutations, and (iii) study the molecular mechanisms of the disease. By RNAi we have identified genes whose partial loss-of-functions could act as modifiers of the disease. Regarding the mechanism of the disease, we work on the hypothesis of genome instability as major driver of s-RP. Mortal germline experiments support this hypothesis that points towards a splicing-independent disease mechanism in which R-loops, PRPF-4 kinase and atl-1/ATR could be involved.

P-72 Uncovering mechanisms of centriole elimination during C.elegans oogenesis

Marie Pierron, Pierre Gönczy

Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, Swiss Federal Institute of Technology (EPFL) Lausanne, Switzerland

The centrosome comprises two centrioles surrounded by pericentriolar material (PCM) and constitutes the principal microtubule organizing center (MTOC) of animal cells. Control of centriole number is critical for bipolar spindle formation and, thus, faithful chromosome segregation. The rules that govern centriole number in proliferating cells must be modified upon cell fusion to avoid supernumerary MTOCs, which would threaten genome integrity. To ensure centriole number reduction at fertilization, centrioles are eliminated from oocytes and endowed to the zygote strictly by the sperm. Despite being a conserved phenomenon in most metazoans, the mechanisms governing centriole elimination during oogenesis remain elusive. To generate insights into this question, we defined the timing and order of disappearance of centriolar proteins during C. elegans oogenesis using dual color confocal imaging of fluorescent SAS-7 together with SPD-2, SAS-4 or SAS-6 in immobilized specimens. We also performed long term time-lapse imaging of fluorescent centriolar proteins in vivo using a microfluidic chamber to determine the duration of the elimination process. In addition, ongoing STED imaging of fixed samples will allow us to determine whether centrioles are eliminated at once of whether intermediary structures can be observed. A study showed that PCM disassembly during Drosophila oogenesis is triggered by Polo-like kinase 1 down-regulation, which then causes centriole elimination. We are likewise testing the potential role of the three worm Polo-like kinases, PLK-1, PLK-2 and PLK-3, as well as the role of PCM in centriole elimination during C. elegans oogenesis.

P-74 Predicting individual differences in viral susceptibility caused by natural genetic variation within species

Lisa van Sluijs[1, 2], Mark G. Sterken[1, 2], Yiru Wang[1], Wannisa Ritmahan[1], Mitra L. Gultom[1], Joost A. G. Riksen[1, 2], Rita J. M. Volkers[1], L. Basten Snoek[1], Gorben P. Pijlman[2], Jan E. Kammenga[1], et al.

[1]Laboratory of Nematology, Wageningen University and Research, Wageningen, The Netherlands

[2]Laboratory of Virology, Wageningen University and Research, Wageningen, The Netherlands

Natural genetic variation within species can underlie different individual susceptibilities upon viral infection. The molecular mechanisms by which genetic variation affects the viral susceptibility are currently poorly understood. Here we use Caenorhabditis elegans as a model organism to identify which polymorphisms alter the viral susceptibility. Moreover, we predict how the molecular mechanisms behind altered susceptibilities may work. The viral susceptibility towards Orsay virus of the commonly used lab strain, N2, is higher than that of the Hawaiian isolate CB4856. The phenotype of N2xCB4856 recombinant inbred strains was obtained by measuring the viral load upon infection and these viral loads were correlated to the genotypes by quantitative trait locus (QTL) mapping. A region on chromosome IV was found to correlate with changes in the viral susceptibility. This QTL region, containing hundreds of candidate polymorphisms, was fine mapped using two introgression line panels. The first introgression line panel contained an introgression of N2 into the genome of CB4856, whereas the second panel contained an introgression of CB4856 into the genome of N2. Using these two panels the QTL region was fine mapped to a region containing about 30 polymorphisms. Using known protein structures we predicted possible effects of candidate polymorphisms. An example is a single nucleotide polymorphism in a conserved region of the known antiviral defence gene cul-6. This polymorphism may be responsible for an altered stability of the SCF complex that targets viral particles for degradation. A causal relationship could be experimentally verified by exchanging the polymorphism of the resistant and susceptible strain, an approach we are currently taking.

P-76 Transcriptional regulatory logic of neuronal ciliogenesis in C. elegans

Rebeca Brocal Ruiz, Nuria Flames

Instituto de Biomedicina de Valencia IBV-CSIC

Cilia are complex evolutionarily conserved subcellular structures that project from cell surfaces to perform a variety of biological roles, ranging from motion to sensation. Cilia are present in multiple cell types and, accordingly, human disorders associated with defects in the function or structure of cilia -known as ciliopathies- comprise a group of multisystemic pathologies. Unfortunately, with an estimated 1 in 1.000 people affected by these diseases, very little is known about the molecular bases of the ciliopathies. In this work we use the simple model organism C. elegans to study the transcriptional regulatory logic controlling ciliogenesis. The RFX transcription factor daf-19 is a direct

activator of many cilium-associated genes in C. elegans. DAF-19 recognizes and binds to cis-regulatory elements, termed X-boxes, to activate the transcription of its target genes. Loss of function of daf-19 results in the lack of expression of those genes and the complete loss of cilia in C. elegans. The role of RFX transcription factors controlling ciliogenesis has been identified in many other organisms, including mammals. To date, RFX members are the only known transcriptional regulators of sensory cilia in any organism, from worms to human. Nevertheless, a number of evidences suggest that daf-19 cannot be acting alone in the regulation of the ciliated program. To identify daf-19 cofactors in C. elegans we are taking an unbiased approach in which we analyse cis-regulatory sequences of cilium-associated genes whose expression is not or only partially affected in daf-19 mutants. To further complement this approach we are also analysing if expression of cilia genes in C. elegans is regulated by a combined action of daf-19 and other transcription factors known to be terminal selectors of non-ciliated features in sub-populations of ciliated neurons in the nematode.

P-78 Notch independent functions of LAG-1/RBPJ in the selection of ADF chemosensory neuron fate

Miren Maicas[1], Ángela Jimeno[1], Mark Alkema[2], Nuria Flames[1]

[1]Developmental Neurobiology Unit, Instituto de Biomedicina de Valencia (IBV-CSIC), Valencia, Spain

[2]Department of Neurobiology, University of Massachusetts Medical School, Worcester, United States.

The nervous system is a very complex organ composed by thousands of different neuron subtypes. This diversity is genetically programmed. Serotonergic neurons are defined by the expression of a battery of enzymes involved in the biosynthesis of serotonin (5HT). C. elegans serotonergic system is composed by three anatomically and functionally different neuronal classes (i.e. NSM, ADF and HSN neurons). Previously, we have determined that each serotonergic neuron subclass uses specific cis-regulatory modules (CRM) that are recognized by different combinations of transcription factors (TF) to induce the expression of the 5HT pathway genes. In this work we have focused on the characterization of the terminal differentiation process of the ADF, the only serotonergic chemosensory neuron in the worm. Mutational analysis of CRMs active in the ADF neuron allowed us to identify functional CSL (CBF-1/RBPJ-κ in human and mouse respectively, Suppressor of Hairless in Drosophila, Lag-1 in C. elegans) binding sites in the regulatory regions of all 5HT pathway genes. RNAi and mutant analysis revealed the requirement of LAG-1 for the correct expression of the 5HT pathway genes as well as for the maintenance of their expression. Our results suggest that LAG-1 acts as a terminal selector for ADF fate. CSL TFs have a conserved role as Notch signalling mediators, however we found that LAG-1 function for ADF specification is independent of Notch signalling. Endogenous lag-1 reporter (by CRISPR tagging) shows ADF expression during all larval and adult stages. Finally, through calcium imaging assays we found that lag-1 is required for the correct ADF activation and function. Our study is the first example of a CSL TF member acting as an activating terminal selector with functions independent of the classical Notch signalling pathway.

P-80 Dithiolic glutaredoxins in Caenorhabditis elegans

Milena Lubisch, Dirk Stegehake, Anne Kaminski, Eva Liebau

Department of Molecular Physiology, Institute of Animal Physiology, University of Muenster, Schlossplatz 8, 48143 Muenster, Germany

Glutaredoxins (GRX) are important components of redox homeostasis. According to their active site, they can be divided into monothiol GRXs and dithiol GRXs. Isoforms of GRX have been implied in quite diverse functions across all kingdoms of life. By regulating the dynamic status of protein S-glutathionylation and thereby altering the activity and function of many target proteins, GRXs are involved in various physiological key roles like redox homeostasis, energy metabolism, protein folding and stability, cytoskeletal assembly and the inflammatory response In C. elegans, five genes encoding for GLRX (in C. elegans GRX is abbreviated GLRX) have been identified. Classification of active site motifs demonstrates the existence of two monothiol (GLRX-3 and GLRX-5) and three dithiol glutaredoxins (GLRX-10, GLRX-21 and GLRX-22). For a better understanding of their role, phenotyping of the three dithiolic GLRX deletion mutants and fluorescent studies have been performed. These show that GLRX-10 and GLRX-22 are constitutively expressed under normal conditions and that under stress conditions the expression of GLRX-22 is reduced. In

contrast GLRX-21 expression is induced under stress conditions. All GLRX knockout mutants show a reduced survival rate under heat stress. For a better understanding of the function of GLRX our main focus lies on the identification of target proteins of S-glutathionylation under normal and stressful conditions. To achieve this, extrachromosomal GFP arrays have been established in the GLRX deletion background. The use of a GFP-Trap® and the substitution of the GLRX N-terminal cysteine residue to a glycine, which prevents the release of the substrate, allows the purification and identification of bound target proteins.

P-82 Inhibition of Sarco-Endoplasmic Reticulum Ca2+ ATPase extends the lifespan in C. elegans worms

PALOMA GARCÍA CASAS, JESSICA ARIAS DEL VAL, PILAR ALVAREZ ILLERA, ROSALBA I FONTERIZ, MAYTE MONTERO, JAVIER ALVAREZ

Institute of Biology and Molecular Genetics (IBGM), Department of Biochemistry and Molecular Biology and Physiology, Faculty of Medicine, University of Valladolid and CSIC, Valladolid, SPAIN.

The sarco-endoplasmic reticulum Ca2+-ATPase (SERCA) refills the endoplasmic reticulum (ER) with Ca2+ up to the millimolar range and is therefore the main controller of the ER [Ca2+] level ([Ca2+]ER), which has a key role in the modulation of cytosolic Ca2+ signaling and ER-mitochondria Ca2+ transfer. Given that both cytosolic and mitochondrial Ca2+ dynamics strongly interplay with energy metabolism and nutrient-sensitive pathways, both of them involved in the aging process, we have studied the effect of SERCA inhibitors on lifespan in C. elegans. We have used thapsigargin and 2,5-Di-tert-butylhydroquinone (2,5-BHQ) as SERCA inhibitors, and the inactive analog 2,6-Di-tert-butylhydroquinone (2,6-BHQ) as a control for 2,5-BHQ. Every drug was administered to the worms either directly in the agar or via an inclusion compound with γ-cyclodextrin. The results show that 2,6-BHQ produced a small but significant increase in survival, perhaps because of its antioxidant properties. However, 2,5-BHQ produced in all the conditions a much higher increase in lifespan, and the potent and specific SERCA inhibitor thapsigargin also extended the lifespan. The effects of 2,5-BHQ and thapsigargin had a bell-shaped concentration dependence, with a maximum effect at a certain dose and smaller or even toxic effects at higher concentrations. Our data show therefore that submaximal inhibition of SERCA pumps has a pro-longevity effect, suggesting that Ca2+ signaling plays an important role in the aging process and that it could be a promising novel target pathway to act on aging.

P-84 The role of neuronal DEG/ENaC ion channel family members in organismal stress responses

Dionysia Petratou[1, 2], Nektarios Tavernarakis[1, 2]

[1]*Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, Heraklion, Crete, Greece*

[2]*Medical School, University of Crete, Heraklion, Crete, Greece*

The integration of sensory stimuli to appropriately modulate behavioral responses to environmental signals, either stressful or not, is critical for organismal survival. The molecular mechanisms that underlie such responses are not fully understood. Dopamine signaling is involved in several forms of behavioral plasticity, in reward processing and in the control of motor output. In Caenorhabtitis elegans the functionality of the dopamine and serotonin pathways can be easily assessed by monitoring specific locomotory responses to environmental food availability cues, termed basal and enhanced slowing. By implementing molecular genetic manipulation technics and behavioral assays we identified three degenerin ion channel proteins to participate in sensory integration through modulation of the dopaminergic pathway. Utilizing advanced imaging technics, we found that degenerins DEL-2, DEL-3 and DEL-4 are expressed in mechanosensory, chemosensory and motor neurons and do not adopt a synaptic localization pattern. These ion channel proteins modulate basal and/or enhanced slowing responses and respond to gustatory stimuli. They act through DOP-2 and DOP-3 dopamine receptors and affect the signaling at the neuromuscular junction. Degenerin effects are largely influenced by stress conditions, such as heat and starvation. Notably, the stress response transcription factors DAF-16/FOXO and SKN-1/Nrf couple degenerin ion channel function to environmental conditions and behavioral output.

P-86 Identification of genetic suppressors of smn-1 in neurodegeneration.

Pamela Santonicola, Ivan Gallotta, Alessandro Esposito, Giuseppina Zampi, Elia Di Schiavi

Institute of Bioscience and BioResources, CNR, Naples, Italy

Smn1 is the gene responsible for Spinal Muscular Atrophy (SMA), a devastating disease characterized by progressive degeneration and death of a specific subclass of motor neurons (MNs). The molecular mechanisms underlying the disease are not completely understood. The lethality associated with loss of function mutations in Smn1 has made the study of its function hard to investigate in any animal model. In C. elegans, the Smn1/smn-1 mutants available present some limitations for manipulation and show no neurodegeneration (Briese et al., HMG, 2009; Sleigh et al., HMG, 2010). To overcome these limits and investigate the role of smn-1 specifically in the nervous system, we used a neuron-specific RNAi strategy to silence smn-1 selectively in the GABAergic MNs (Gallotta et al., HMG 2016; Esposito et al., Gene, 2007). These animals, viable and fertile, present an age-dependent degeneration of MNs that results in neuronal cell death and altered backward movements. The neurodegenerative phenotypes shown by the MNs silencing of smn-1 can be modulated by Smn1 interactors identified in other species, such as Plastin3/plst-1 and WDR79/tcab-1 (Gallotta et al., HMG 2016; Di Giorgio et al., Neurobiol Dis 2017). We are now using this model as a tool to identify, by forward and reverse genetics approaches, genes that interact with smn-1 and that once mutated fully rescue the degeneration. After excluding genes impairing the RNAi machinery or involved in apoptotic death execution, we are focussing on the genes fully protecting neuronal integrity and survival in early phases. We will present the most interesting ones, that are also able to rescue the locomotory function.

P-88 Robustness in the transcriptional programs that maintain neuronal cell fates

Konstantina Filippopoulou, Guillaume Bordet, Carole Couillault, Vincent Bertrand

Institut de Biologie du Développement de Marseille, CNRS, Aix-Marseille University

During nervous system development, neurons with specific functions are generated. The acquisition and maintenance of their differentiated state are achieved by terminal transcription factors (TFs). These TFs are expressed throughout the life of the neurons and often autoregulate their own expression via positive feedback loops. To produce an invariant output this transcriptional network has to be strictly controlled and robust to internal and external perturbations. The objective of this study is to determine how the specific fate of a neuron is maintained during the life of the animal in a reliable manner despite gene expression noise. To address this question, we use the well characterized differentiation network of the AIY interneurons in C. elegans. In our lab, it has been shown that the expression of this network's terminal TFs is noisy and that chromatin factors, members of the PRC1 complex, display a protective role on the fate of AIY neurons. A stochastic and progressive loss of the identity of AIY neurons is observed when components of the PRC1 complex are mutated. In the present study, we show that PRC1 also preserves the identity of AIY neurons against environmental perturbations, and we are currently characterizing the underlying mechanism.

P-90 The role of developmental genetic architecture in shaping evolutionary trends

Joao Picao-Osorio[1], Christian Braendle[2], Marie-Anne Félix[1]

[1]*Institut de Biologie de l'Ecole Normale Supérieure (IBENS) ; CNRS UMR 8197; ENS; Paris, France*

[2]*Institut de Biologie Valrose; CNRS UMR7277; Nice, France*

Random mutation of the genotype does not generate random phenotypic variation because development biases the mutationally inducible phenotypic spectrum. Therefore, understanding such biases in the introduction of phenotypic variation is essential to reveal which phenotypes can be explored and selected in the evolutionary process. Whether such developmental genetic biases in the construction of phenotypic variation influence evolutionary trends is poorly understood. Here we address this problem using the homologous cellular framework of vulval precursor cells in two clades of nematodes that have divergent evolutionary trajectories of cell fate variation. In Caenorhabditis species, among the six vulva precursor cells (VPC) termed P3.p to P8.p, only P3.p cell fate shows significant evolutionary variation within and among species. In contrast, in Oscheius species (sister genus) evolutionary variation of cell fates is highest in different precursor cells, i.e. P4.p and P8.p. We will probe the role of

developmental constrains in shaping evolutionary trends of nematode vulva cell fate variation. For this, we will access the mutationally accessible phenotypic spectrum (i.e. mutational variance) using a combination of random mutagenesis and quantitative genetics. First, we will generate eight panels of random mutant lines in wild isolates of Caenorhabditis and Oscheius to quantify the mutability of VPC fates across micro and macro-evolutionary scales. Second, we will compare this mutational variance with the natural cell fate variation at different evolutionary scales. This project will lead us to causally connect developmental mutability and evolutionary trends at the single-cell level.

P-92 The Pre-Replication Complex Governs the Invasive Cell Fate of the Caenorhabditis Elegans Anchor Cell in a Replication-Independent Manner

Evenlyn Lattmann[1, 2], Ting Deng[1], Vibhu Prasad[1], Charlotte Lambert[1], Michael Daube[1], Ossia Eichhoff[2], Urs Greber[1], Reinhard Dummer[2], Mitch Levesque[2], Alex Hajnal[1], et al.

[1]IMLS, University of Zurich

[2]Dermatology, University Hospital Zurich, Zurich, Switzerland

Insights into the mechanisms of cell invasion, the first step of metastasis formation, holds great promise for the development of new targeted therapies of malignant cancers. Here, we used the Caenorhabditis elegans anchor cell invasion model to gain further knowledge of invasion through basement membranes. By screening the C. elegans orthologs of genes highly expressed in invasive human melanoma cells, we identified five pre-replication complex components, mcm-7, cdc-6, cdt-1, orc-2 and orc-5 that regulate basement membrane breaching by the anchor cell. Despite the well-described function of the pre-replication complex in origin of replication licensing for DNA replication in S-phase, we found that mcm-7 regulates invasion in the G0/G1 arrested anchor cell. Furthermore, components of the DNA replication machinery (e.g. GINS complex, cdc-7) are not required for anchor cell invasion, suggesting a DNA replication-independent function of the pre-replication complex. RNAi-mediated knock-down of mcm-7 interferes with invadopodia formation and reduces the expression of the extracellular matrix proteins zmp-1, him-4 and protocadherin cdh-3. Preliminary data suggest that the reduced expression of these pro-invasive genes is a consequence of decreased egl-43L transcription, while mcm-7 RNAi does not affect hlh-2 or fos-1 expression. In the human melanoma cell line A375, knockdown of MCM7 by siRNA causes an accumulation of cells in the G1 phase of the cell cycle with unchanged expression of CCND1. Currently, we are investigating whether pre-replication complex components regulate the invasive behaviour of human melanoma cells. In summary, we propose that components of the pre-replication complex have adopted a replication-independent function to regulate anchor cell invasion.

P-94 Intestinal polarity is maintained by V0-ATPase-dependent apical sorting in C. elegans

Aurelien Bidaud-Meynard, Ophélie Nicolle, Gregoire Michaux

Univ Rennes, CNRS, IGDR - UMR 6290, F-35000 Rennes, France

Food absorption relies on the strong polarity of intestinal epithelial cells and the array of microvilli forming a brush border at their luminal (apical) pole. Some rare genetic enteropathies, characterized by brush border defects leading to food malabsorption, are caused by mutations in genes coding for membrane traffic factors (i.e. Myo5B, STX3 in microvillus inclusion disease/MVID; Munc18-2 in FHL-5). We and others have recently shown that enterocytes from MVID patients display mispolarized polarity modules and brush border structural proteins. Despite this functional link, little is known about the interaction between membrane traffic, polarity and brush border components, especially in vivo. Combining genetics and in vivo super-resolution imaging tools in C. elegans intestine model, we uncovered that the transmembrane sector (V0) of the V-ATPase plays a major role in the maintenance of the intestinal polarity. Indeed, V0-ATPase silencing leads to the basolateral mislocalization of apical polarity determinants (i.e. CDC-42/PAR-3/PAR-6/PKC-3 module) and brush border components (i.e. ezrin/ERM-1) as well as microvilli morphological defects and formation of presumptive microvillus inclusions in the cytoplasm. This domain has been previously implicated in the fusion of vesicles with the plasma membrane, independently of the H+ pump function of the whole V-ATPase complex (V0+V1 domains). Further characterization showed that V0-ATPase: 1) specifically controls a trafficking step involving RAB-11-positive apical recycling endosomes and 2) genetically and

functionally interacts with lipids biosynthesis pathways. Notably, polarity loss, microvillus inclusions and RAB-11-endosomes failure are the hallmarks of MVID and FHL-5 enteropathies. Thus, besides the discovery of a new role for the V0-ATPase in epithelial polarity maintenance, we propose to use the C. elegans intestine as an in vivo model to better understand the molecular mechanisms of rare genetic enteropathies.

P-96 Acute drug responses in C.elegans reveal complex novel biology

Andy Fraser, Mark Spensley, Sam Dell Borrello, Margot Lautens, Taylor Davie
The Donnelly Centre, Toronto

Many drugs act very rapidly - they can turn on or off their targets within minutes in a whole animal. What are the acute effects of drug treatment and how does an animal respond to these? We developed a simple image-based assay to measure the acute effects of drugs on C.elegans movement - this can track up to 5000 worms simultaneously at ~1 second resolution and can thus measure acute drug responses at high throughput. We examined the effects of a range of compounds including neuroactive drugs, novel compounds, toxins, and environmental stresses on worm movement. We find that many treatments show complex acute responses - an initial phase of rapid paralysis is followed by one or more recovery phases. The recoveries are not the result of some generic stress response but are specific to the drug. We also find that acute responses can vary greatly across development and that there is extensive and complex natural variation in acute responses. Acute responses are sensitive probes of the ability of biological networks to respond to rapid changes in gene activity due to drug treatment and these responses can reveal the action of unexplored pathways. We used our assay to examine the effects of metabolic inhibitors on worm movement and find that we can drive C.elegans to use a highly unusual anaerobic metabolic pathway that is used by parasitic helminths in their human hosts. This allows us to do both genetic and drug screens to dissect this pathway and to identify new treatments for the helminth infections that affect up to 2 billion humans. I will present the assay, the diversity of acute responses that we see, and then focus on the dissection of this anaerobic metabolic pathway.

P-98 From the transcriptome to the metabolome: A systems view on the impact of the mitochondrial prohibitin complex during aging.

Artur Bastos Lourenço[1], Francisco J García-Rodríguez[1], Karl Burgess[2], Christoph Kaleta[3], Marta Artal Sanz[1]
[1]*Andalusian Centre for Developmental Biology (CABD), CSIC-Universidad Pablo de Olavide*
[2]*Glasgow Polyomics Facility, University of Glasgow*
[3]*Research Group Medical Systems Biology, Institute of Experimental Medicine, Christian-Albrechts-University Kiel*

The mitochondrial prohibitin (PHB) complex, composed of PHB-1 and PHB-2, is a context-dependent modulator of longevity. Specifically, PHB deficiency shortens the lifespan of wild type Caenorhabditis elegans, while it dramatically extends lifespan under compromised metabolic conditions, as is the case of insulin receptor daf-2(e1370) mutants. This extremely intriguingly phenotype has been linked to alterations in mitochondrial function and fat metabolism. To understand the impact of PHB depletion on aging, we gather RNA-seq and metabolomics data from wild-type and daf-2(e1370) animals in the presence as well as absence of PHB at the young adult stage and in the course of aging. In parallel, oxygen consumption rates were measured. The gene expression data was integrated into a genome-scale metabolic network of C. elegans for in silico identification of specific metabolic signatures of aging and identification of key enzymes controlling these signatures. This extraordinary detailed dataset gives us valuable insights on the impact of the PHB complex during aging. It reveals that PHB depletion has a major effect on metabolism, which is much more pronounced in wild type animals compared to daf-2 mutants. In particular, we observe a broad effect on carbohydrate and amino acid metabolism and, importantly, a differential effect on redox homeostasis. We hypothesised that PHB might impact the mitochondrial respiratory chain (MRC), resulting in differential energy mobilization and efficiency. We observe by Blue-native PAGE analysis of enriched mitochondrial fractions that PHB has a differential effect on the MRC of wild type and daf-2 mutants. Consistently, oxygen consumption rate measures points to a differential effect of PHB depletion during aging. These new findings will be discussed in the scope of the opposing longevity phenotype of PHB depletion.

P-100 Activation of insulin signaling induces cell divisions in Caenorhabditis elegans L1 arrest.

Ian Chin-Sang, Shanqing Zheng, Jeffrey Boudreau

Department of Biology, Queen's University, Kingston ON, Canada

Caenorhabditis elegans is an excellent model to study the molecular mechanisms that transmit food signals to growth and development. C. elegans that hatch in the absence of food stop their development in a process called L1 arrest. Here, we report that mutations in daf-18/pten or aak-2/ampk cause postembryonic neuronal Q cell divisions and migrations during L1 arrest as if the worms were in a fed state. DAF-18/PTEN functions cell autonomously, independent of DAF-16/FOXO, in insulin/IGF-1 signaling (IIS) to stop Q cells divisions and migrations during L1 arrest and human PTEN can functionally replace DAF-18. We find that insulin-like peptides (INS) act cell non-autonomously to promote cell divisions during L1 arrest, and DBL-1/BMP functions upstream of the INS peptides. Chemical screening and genetics provide evidence that energy sensor, AMPK, works downstream of IIS and blocks the function of Protein Phosphatases, PP2As. Further, we show that in the absence of DAF-18/PTEN or AAK-2/AMPK, PP2As promote Q cell divisions in L1 arrest by activation of MPK-1/ERK via LIN-45/RAF in the MAPK signaling pathway. We have used this new L1 arrest Q cell division phenotype, as well as other IIS phenotypes as an in vivo readout of IIS. We have systematically overexpressed all 40 Insulin like peptides in the C. elegans nervous system for their ability to activate or inhibit the DAF-2 insulin receptor. We have identified in vivo functions for 30 of the 40 INS and will use this data to determine what makes an INS peptide and activator or inhibitor of the DAF-2 insulin like receptor.

P-102 Identification of Heterotrimeric G-proteins Involved in the Modulation of the Exogenous RNA Interference Response by IP3 Signalling in C. elegans

Alice Rees, Howard Baylis

University of Cambridge, Department of Zoology

Previous work from our lab revealed that the IP3 signalling pathway regulates the exogenous RNA interference (RNAi) response in C. elegans (Nagy et al, 2015). IP3 is a common second messenger involved in the regulation of a number of key functions (Baylis and Vázquez-Manrique, 2012). IP3 is produced by the hydrolysis of the phospholipid PIP2 by phospholipase C (PLC), in response to activation by G-protein coupled receptors or receptor tyrosine kinases. Activation of the IP3 Receptor (IP3R) leads to release of intracellular calcium stores. C. elegans has been a popular system for the study and exploitation of RNAi owing to a robust and systemic response to exogenous dsRNA. Mutation of components of the IP3 signalling pathway leads to an altered response to RNAi induced by feeding or transgenes. Mutants with reduced levels of IP3 signalling such as loss-of-function of IP3R (ITR¬-1), or the PLCβ (EGL-8) result in an enhanced RNAi response, whilst loss-of-function of IPP-5, leading to increased levels of IP3 results in a reduced RNAi response. In order to identify the potential upstream activators of EGL-8 involved in the modulation of the RNAi response we screened a number of different Gα subunit mutants for an altered response to RNAi by feeding. Loss of function mutants in the canonical activator of PLCβ (EGL-8), Gαq (EGL-30) do not show an enhanced RNAi response. However, loss of function mutation of the Gαo homologue (GOA-1) leads to an enhanced RNAi response, suggesting that it may be an activator of EGL-8 in the IP3 signalling pathway regulating the RNAi response. We aim to demonstrate this via epistasis analysis. We have also screened a number of RGS proteins, known to have a role in the regulation of GOA-1 signalling, for an altered RNAi response in order to further understand the signalling network regulating this response.

P-104 EXC-4/CLIC and G-protein signaling during tubulogenesis in *C. elegans*

Anthony Arena, Dan Shaye

University of Illinois-Chicago. Dept. of Physiology and Biophysics. Chicago, IL. USA

Biological tube formation (tubulogenesis) is a key process during vascular development and angiogenesis. The chloride intracellular channel (CLIC) family was first implicated in tubulogenesis by the discovery that EXC-4, a worm CLIC, is required for *C. elegans* excretory canal (*Ce*EC) tubulogenesis. Following this discovery, it was shown that two mammalian CLICs, CLIC1 and CLIC4, are expressed in vascular endothelial cells and are required for angiogenesis.

EXC-4 is constitutively localized to the apical plasma membrane in the *Ce*EC and this localization is critical for function. Human CLIC1 can rescue *exc-4* null *(0)*, but only when targeted to the apical membrane, demonstrating conservation of function and the importance of membrane targeting. In contrast to EXC-4, human CLICs accumulate in the cytoplasm, but are transiently recruited to the plasma membrane upon G-protein-coupled receptor (GPCR) activation. This transient localization appears to depend on the GPCR effector RhoA. To further understand the conserved role and regulation of CLICs in tubulogenesis we want to know whether EXC-4 activity and localization is regulated by GPCR and Rho-family signaling in *C. elegans*. Previously-described *exc-4* alleles are nulls, exhibiting strong and fully-penetrant cystic phenotypes. As such, we cannot use them to analyze genetic interactions with mutants in GPCR and Rho-family signaling, because enhancement is not possible and suppression may not occur if *exc-4* is completely absent. By scanning the Million Mutation Project we found mutations that 1) affect conserved EXC-4 residues, 2) exhibit *Ce*EC phenotypes, 3) are recessive, and 4) fail to complement *exc-4(0)*. We are currently analyzing genetic interactions between these new hypomorphic *exc-4* alleles and mutations that affect GPCR and Rho-family signaling. Finally, to further understand the regulation of EXC-4 localization we are examining the effect of mutants in GPCR-signaling, and in Rho-family members, as well as the effect of mutations in conserved residues, on EXC-4 accumulation.

P-106 Regulation of Caenorhabditis elegans neuronal function by the putative oxaloacetate decarboxylase FAHD-1

Giorgia Baraldo[1], Hildegard Mack[2], Pidder Jansen-Dürr[1]

[1]*Institute for Biomedical Aging Research, Department for Molecular Biosciences Innsbruck, University of Innsbruck, Austria;*

[2]*Institute for Biomedical Aging Research, Department for Biochemistry and Genetics of Aging Innsbruck, University of Innsbruck, Austria;*

Fumarlyacetoacetatehydrolasedomain containing protein 1 (FAHD1) is part of an evolutionary conserved family of prokaryotic proteins that were recently also found in eukaryotes. FAHD1 is an oxaloacetate decarboxylase, which turns oxaloacetate into pyruvate in human cells. Through its activity, the protein can influence the metabolic rate by regulating the amount of oxaloacetate available for the Krebs- cycle. Genetic studies revealed a single FAHD1 orthologue in C. elegans. Specifically, fahd-1 deletion mutants show a deleterious phenotype characterized by an impaired locomotion and a downregulated egg-laying rate, which leads to a longer storage of the eggs in the nematode´s uterus. Additionally, when exposed to serotonin to force egg-laying release, the fahd-1 deletion mutant shows resistance to the treatment. Here we sought to investigate the underlying molecular mechanisms that see loss of fahd-1 involved in this striking phenotype. Since the main drivers of locomotion and egg-laying are neurons and muscle cells, tissue specific rescue stains were generated to understand the role of fahd-1 in C. elegans physiology. We found that re-expressing fahd-1 in neurons is sufficient to fully restore the normal egg-laying behavior and partially the normal locomotion. Furthermore, exposure of fahd-1 deletion mutants to dopamine shows a similar attenuation effect for the egg-laying defect. Based on our observations we suggest that fahd-1 modulates neuronal activity in nematodes and possibly, constitutes a link between metabolism and neurological dysfunction.

P-108 Cell-cell fusion of chemosensory neurons alters the animal's response to odours.

Rosina Giordano-Santini[1], Eva Kaulich[1, 2], Massimo A. Hilliard[1]

[1]*Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, Brisbane, Australia*

[2]*Division of Neurobiology, MRC Laboratory of Molecular Biology, Cambridge, UK.*

The neuron theory proposed by Ramón y Cajal over a century ago states that neurons are individual cells, rather than a reticulum as hypothesized by Camillo Golgi. Despite Cajal's model being confirmed in every species studied, mounting evidence suggests that in certain conditions, such as following viral infection, injury or environmental stress, neurons can lose their individuality by undergoing cell-cell fusion. The molecular causes of these events are

unknown, and the functional consequences are still poorly understood. We hypothesize that neuron-neuron fusion will affect the neural circuit wiring, leading to an altered behavioural output. To address this idea, we use the C. elegans chemosensory neurons as a model system. We focus on two pairs of chemosensory neurons in the head the animal, AWCon and AWCoff that mediate attraction to odours, and AWB left and right that mediate repulsion to odours. In other tissues in which cell-cell fusion occurs during normal development, specific molecules known as fusogens mediate the merging of the adjacent plasma membranes. We overexpressed the C. elegans fusogen EFF-1 in the AWCs and AWBs neurons to induce fusion between these cells. We found that fusion between neurons mediating attraction to odours (AWCon and AWCoff) does not cause a change in the animal's behaviour. On the contrary, fusion between neurons with opposite functions, such as AWCs and AWBs neurons, results in loss of both attraction and repulsion. We are currently investigating if fusion changes the neural fate, or if it causes a short-cut between the attractive and repulsive circuits. To our knowledge, this is the first study that shows how neuron-neuron fusion affects the function of the nervous system in vivo. We propose neural fusion as a synthetic biology approach to study neural circuits and how changes in connectivity modify behaviour.

P-110 The TRIM32 protein GRIF-1 controls developmental proteolysis of GLD-2 cytoPAP

Tosin Oyewale[1, 2], Christian Eckmann[1]

[1]*Institute of Biology, Division of Genetics, Martin Luther University of Halle-Wittenberg, Germany*

[2]*DFG-GRK1591, Martin Luther University of Halle-Wittenberg, Germany.*

A common feature of early embryogenesis in many organisms is transcriptional repression. Therefore, a large number of RNA regulators, such as RNA modifying enzymes and other mRNA associated factors control early development at the post-transcriptional level by regulating the fate of maternal mRNA. While several works have addressed how these RNA regulators act during development, little is known about how their maternal expression is regulated. In studying the embryonic expression pattern of C. elegans cytoplasmic polyA-polymerase (cytoPAP) GLD-2, we identified GRIF-1, a putative GLD-2-interacting ring finger domain-containing ubiquitin ligase. In co-immunoprecipitation experiments using embryonic extracts, we found that GRIF-1 associates with GLD-2 cytoPAP in an RNA-independent manner, and in Y2H binding test, we identified the germline-specific N-terminal domain of GLD-2 as important for a direct interaction with GRIF-1. Consistent with a regulatory role of GLD-2's N-terminal domain, its removal does not affect GLD-2 expression and functions in the adult. Furthermore, we found that GRIF-1 protein is exclusively expressed in embryonic germ cells and appears just prior to GLD-2's disappearance in primordial germ cells. Importantly, when we compromised grif-1's functions, GLD-2 cytoPAP expression was prolonged compared to wild-type embryos, and these embryos produced animals with a mortal germline phenotype: germ cells are able to maintain their identity and proliferate across some generations before losing proliferation capacity and undergoing cell death in a manner that is partially dependent on apoptosis; a phenotype that is exacerbated by heat stress. Taken together, GRIF-1 is a germ cell-specific, maternally expressed factor that regulates embryonic GLD-2 cytoPAP expression presumably via ubiquitin-mediated degradation to increase germ cell fitness across generation.

P-112 Differences in Endogenous Proteasome Expression in C. elegans

Elisa Mikkonen[1], Caj Haglund[1, 2], Carina I Holmberg[1]

[1]*Research Programs Unit, Translational Cancer Biology Program, University of Helsinki, Helsinki, Finland*

[2]*Department of Surgery, University of Helsinki and Helsinki University Hospital, Helsinki, Finland*

Regulated protein turnover is critical for normal cell function. The ubiquitin-proteasome system (UPS) is responsible for degradation of most soluble and misfolded intracellular proteins. Dysfunctions in this system are linked to various aging-related disorders including neurodegenerative diseases and various types of cancer. Regulation of the UPS and proteasome function in a multicellular organisms is a complex and still fairly unknown process. We have previously shown with our fluorescent UPS reporters that UPS-mediated protein degradation varies in a cell-type and age-dependent manner in live C. elegans [1, 2]. Here, we have set up immunohistochemistry on formalin-fixed, paraffin-embedded C. elegans to facilitate semi-quantitative studies on the endogenous expression of the proteasome at a tissue- and cell-level resolution. We show that the proteasome immunoreactivity pattern differs between cell types

and within subcellular compartments in adult wild-type C. elegans [3]. Interestingly, long-lived daf-2(e1370) mutants with impaired insulin/IGF-1 signaling (IIS) display similar proteasome tissue expression as aged-matched wild-type animals, although these animals have increased proteasome activity for example in intestinal cells. We also revealed that widespread knockdown of proteasome subunits by RNAi results in tissue-specific changes in proteasome expression instead of a uniform response [3]. Our data highlight the importance of implementing methods that capture changes in protein expression and activity occurring at cell or tissue resolution level in a multicellular organism. 1. Hamer G, Matilainen O, Holmberg CI. A photoconvertible reporter of the ubiquitin-proteasome system in vivo. Nat. Methods. 2010;7: 473-478. 2. Matilainen O, Arpalahti L, Rantanen V, Hautaniemi S, Holmberg CI. Insulin/IGF-1 signaling regulates proteasome activity through the deubiquitinating enzyme UBH-4. Cell. Rep. 2013;3: 1980-1995. 3. Mikkonen E, Haglund C, Holmberg CI. Immunohistochemical analysis reveals variations in proteasome tissue expression in C. elegans. PLoS One. 2017;12: e0183403.

P-114 A transcription factor collective defines the HSN serotonergic neuron regulatory landscape

Carla Lloret-Fernández[1], Miren Maicas[1], Carlos Mora-Martínez[1], Alejandro Artacho[2], Ángela Jimeno-Martín[1], Laura Chirivella[1], Peter Weinberg[3], Nuria Flames[1]

[1]*Developmental Neurobiology Unit. Instituto de Biomedicina de Valencia IBV-CSIC, Valencia*

[2]*Departamento de Genómica y Salud, Centro Superior de Investigación en Salud Pública, FISABIO, Valencia*

[3]*Department of Biochemistry and Molecular Biophysics, Howard Hughes Medical Institute, Columbia University Medical Center, NY, USA*

The identification of rules underlying transcriptional cis-regulatory codes in different cell types remains a challenging task. To address how particular combinations of transcription factors (TFs) specify neuronal fates, we take advantage of the simple model Caenorhabditis elegans and study the evolutionary conserved serotonergic system. We have identified a collective of six TFs that act at the terminal differentiation level to orchestrate the activation of the transcriptome of the HSN serotonergic neuron (Lloret et al. eLife in press). These TFs, that we term the HSN TF collective, act directly upon the genes they regulate and show enhancer-context dependent synergistic behaviors. Remarkably, among all C. elegans neurons, the HSN transcriptome most closely resembles that of mouse serotonergic neurons. Mouse orthologs of the HSN regulatory code are known regulators of mammalian serotonergic differentiation programs and we demonstrate that they can functionally substitute their worm counterparts, suggesting they share deep homology. Additionally, we found that the presence of TF binding sites clusters for the HSN TF collective configure a regulatory signature that allows for de novo identification of HSN neuron functional enhancers. Our bioinformatics prediction shows a 40% success rate, indicating that the presence of the HSN TF regulatory signature per se is not sufficient to distinguish between HSN active or inactive enhancers. Here we will present our new, unpublished data, on additional features of the HSN regulatory signature (additional TF binding motifs and specific syntax rules) that are present in HSN functional enhancers.

P-116 C.elegans progenitor cell fate control through the EGR-type transcription factor LIN-29

Chiara Azzi[1, 2], Florian Aeschimann[1], Anca Neagu[1], Helge Grosshans[1]

[1]*Friedrich Miescher Institute for Biomedical Research, Maulbeerstrasse 66, 4058 Basel, Switzerland*

[2]*University of Basel, Faculty of Science, 4056 Basel, Switzerland*

EGR-type transcription factors are regulators of stem cell self-renewal and differentiation in animals. Caenorhabditis elegans LIN-29/EGR, together with its NAB orthologous co-factor MAB-10, regulates the larva-to-adult (L/A) switch, a developmental event characterized by termination of self-renewal of seam cells, skin progenitor cells. Other events of the L/A switch are fusion of the seam cells, production of an adult cuticle and termination of the molting cycle. We found that different isoforms of LIN-29 have different expression patterns and are differentially regulated: the RNA binding protein LIN-41 inhibits expression of lin-29A and mab-10, while the transcription factor HBL-1 inhibits expression of lin-29B. Moreover, our results reveal that different isoforms of LIN-29 control distinct L/A switch events, suggesting that although normally tightly coupled, terminal cell differentiation and cell cycle exit are distinct, independently controlled processes in this system. With our results we could update the model of the heterochronic

pathway: hbl-1 and lin-41 constitute two parallel arms of the pathway acting on lin-29B vs. lin-29A and mab-10, respectively. The two parallel arms converge on the execution of the L/A switch, complete execution of which requires expression of both LIN-29 isoforms and their co-factor MAB-10 in the epidermis.

P-118 C elegans high content behavioural screening for drug repositioning

Ida Barlow[1, 2], Adam McDermott-Rouse[2], Oliver Howes[1, 3], Andre Brown[1, 2]

[1]*Imperial College*

[2]*MRC London Institute of Medical Sciences*

[3]*Kings College London*

Genetic screens in C elegans have shown that mutations in similar genes result in shared behavioural phenotypes due to modulation of shared molecular pathways (Brown et al. 2013; Yemini et al. 2013). However, it is not known whether similar drugs used to treat comparable diseases act through shared molecular pathways and result in common phenotypes. Furthermore, over 26,000 drugs are clinically approved for disease treatment (https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm), but our mechanistic understanding for many of these compounds is lacking. Therefore, it would be advantageous to gain a better understanding of clinically approved drugs in order to provide a novel insight into disease aetiology and drug mode of action to propose compounds for repositioning. Using high-resolution multi-animal tracking and quantification of C elegans posture and behaviour, we have tested a pilot panel of 7 antipsychotic drugs and demonstrated that diverse and common molecular structure and pharmacology can be represented by distinct high-dimensional behavioural phenotypes. Targeted modulation of molecular pathways and neuronal networks is indicated by dose-dependent modulation of multi-dimensional behavioural phenotypes. Using machine learning we have identified a unique multi-parameter behavioural profile for the most effective antipsychotic drug on the market, clozapine, which will provide a reference for the discovery of other compounds that may be clinically relevant for the treatment of psychoses. Therefore, this pilot screen has demonstrated a proof-of-concept for implementing a C elegans behavioural screen of over 2000 compounds using a newly developed high-throughput behavioural rig. Our high-resolution, multi-well tracking arena will be able to simultaneously and quantitatively track >200 worms in 50 distinct conditions to enable us to screen a large library of compounds at multiple doses. The resultant comprehensive phenotypic database will provide a unique insight into modulation of C elegans behaviour and drug pharmacology, which will be useful in the identification of candidates for drug repositioning.

P-120 Stress-induced cellular surveillance and behavioural responses exhibit a complementary pattern in Caenorhabditis elegans

Eszter Gecse, Beatrix Gilányi, Márton Csaba, Csaba Sőti

Semmelweis University, Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Budapest, Hungary

Tissue damaging stresses evoke an evolutionary conserved protective response from the cellular through the organismal level. For instance, disruption of vital cellular functions induces xenobiotic and immune-related molecular responses and aversive behaviour of the perceived danger. However, whether and how the cellular and behavioural responses are co-ordinated is unclear. In this study, we investigate this phenomenon by exposing C. elegans to various toxic insults and inactivation of essential gene functions and analyze the relationship between phenotypic changes, induction of cellular stress and detoxification reporters and the aversive behavioural response. We observed that exposure to either the terminal oxidation inhibitor antimycin or mitochondrial superoxide inducer paraquat in the bacterial lawn from hatching resulted in developmental retardation and aversive behaviour in a dose-dependent manner. Similarly, food leaving and/or disturbed development was detected in response to the proteostasis regulator daf-21/hsp90 and proteasome subunit rpt-5 RNAi silencing. At the molecular level, antimycin treatment induced the expression of the cyp-35b::gfp phase I detoxification enzyme reporter, but no induction of the phase II gst-4::gfp reporter was detected. In contrast, paraquat treatment induced the expression of gst-4::gfp, whereas cyp-35b::gfp was not induced. Further, both antimycin and paraquat induced the mitochondrial stress marker hsp-6::gfp expression to different extents. Intriguingly, worms that did not avoid the food lawn exhibited a

significantly higher expression of the abovementioned molecular surveillance markers. We conclude that different dangerous insults result in similar phenotypic and behavioural changes and overlapping, yet differential molecular surveillance responses. There is a heterogeneity and an inverse correlation between the molecular and behavioural protective responses. Further experiments are under way to understand the differential responses and the mechanisms behind.

P-122 Effects of asymmetric cell division on regulation of cell migration

Erik Schild[1], Shivam Gupta[2], Andrew Mugler[2], Hendrik Korswagen[1]

[1]Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences and University Medical Center Utrecht, Uppsalalaan 8, 3584 CT Utrecht, the Netherlands

[2]Department of Physics and Astronomy, Purdue University,West Lafayette, Indiana 47907, USA

The migration of QR neuroblast descendants in C. elegans is regulated by multiple Wnt signaling pathways. In one of the descendants, QR.pa, a phase of long-range migration is ended when canonical Wnt/β-catenin signaling is activated. This activation is achieved by time-dependent upregulation of the Wnt receptor mig-1. Interestingly, the activation of canonical Wnt signaling coincides with a mitotic division of QR.p. This division results in a small, apoptotic daughter cell QR.pp, and the larger QR.pa, which will later produce two neurons. mig-1 levels start rising before division, and increase further in QR.pa. The Mugler group has created a mathematical model for the regulation of time-dependent expression of mig-1 in QR.p and its descendants. The model is used to predict in what way mig-1 expression is most likely regulated. However, the model depends on assumptions about currently unknown aspects of QR.p division. One of these is whether or not the asymmetry of QR.p division impacts mig-1 regulation, and thus migration. Determining this would improve the accuracy of the model. To assess the impact of asymmetric cell division, we will study the mig-1 levels in three different C. elegans mutants. First, we will study a mutant strain in which QR.pp cannot undergo apoptosis, which enables us to study mig-1 expression dynamics in the normally apoptotic QR.p descendant QR.pp. Second, we will study a mutant strain in which QR.p divides symmetrically, causing QR.pp to adopt the fate of QR.pa and produce extra neurons. Last, we are generating a strain in which QR.p is prevented from dividing entirely by using the auxin inducible degradation system. Together, the expression of mig-1 observed in these experiments will indicate whether asymmetric cell division is functionally relevant for the switch in Wnt signaling response that terminates QR descendant migration.

P-124 Protective effect of plant hormones cytokinins in Caenorhabditis elegans

Alena Kadlecová[1], Tomáš Jirsa[1], Ondřej Novák[1], Martin Hönig[2], Lucie Plíhalová[2], Karel Doležal[2], Miroslav Strnad[1], Jiří Voller[1]

[1]Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany ASCR, Palacký University, Šlechtitelů 27, 78371, Olomouc, Czech Republic.

[2]Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Šlechtitelů 27, CZ-783 71 Olomouc, Czech Republic.

For decades, researchers used a model nematode Caenorhabditis elegans for studying pathways, mutations and interventions that can influence aging. Many of these processes are conserved from yeast to mammals. C. elegans is also routinely utilized for studying compounds with anti-aging and protective activity. Such compounds can then find application in cosmetics or potentially in medicine, where they can be used to increase the quality of life of elderly patients. In this study, we used C. elegans to test the protective effect of plant hormones cytokinins. Cytokinins, N6- substituted derivatives of adenine, are responsible for a wide range of plant processes, including cell division, differentiation, development and leaf senescence. However, for some of them also a protective activity in animal models was described. We investigated a series of naturally occurring cytokinin bases and their (semi)synthetic derivatives for their lifespan-prolonging effect and ability to increase stress resistance in the nematode. Three natural compounds prolonged the lifespan of the nematode. Kinetin, which was selected as a pilot compound for follow-up experiments, was effectively absorbed and metabolized by the worms. It also increased the resistance of C. elegans to oxidative and heat stress. The effect of kinetin was dependent on the presence of reactive

oxygen species, and independent on insulin/insulin-like signaling pathway. Following screening of structurally related derivatives from our library revealed several more potentially active compounds.

P-126 The interneuron RIS and lethargus in C. elegans

Elisabeth Maluck, Henrik Bringmann
Max Planck Institute for Biophysical Chemistry Göttingen, Germany

For the majority of organisms having a nervous system, sleep is an essential behaviour. Therefore one assumes sleep mechanisms to be conserved among species. The nematode C. elegans offers the great opportunity to gain insights into sleep regulation and function in a simple neuronal environment and to later on confer the knowledge on higher organisms. In C. elegans sleep occurs during development, in response to diverse stresses or after the worms were fed with high quality food (satiety quiescence). This poster focuses on the developmental sleep (lethargus). Within the C. elegans neuronal network the RIS neuron is especially important for the regulation of developmental sleep. RIS is mostly inactive in wake animals but it undergoes a strong activation around sleep onset. Previous studies classified it to be GABAergic and peptidergic. In our lab we could show that RIS releases the neuropeptide FLP-11 to induce sleep by causing the immobilization of worms. Furthermore the FLP-11 promotor turned out to be cell-specific for RIS. That allowed us to generate optogenetic tools using ReaChR and ArchT to specifically wake worms up or make them fall asleep. While artificially manipulating sleep/wake states, we monitored neuronal activities of RIS upstream and downstream neurons and found out that RIS activity seems to be neuronally regulated. Additionally, as it is already known for sleep in general, RIS activity seems to be exposed to a homeostatic regulation.

P-128 The role of the HOX protein LIN-39 in the regulation of VPC proliferation

Svenia Heinze, Alex Hajnal
Institute of Molecular Life Science, University of Zurich

The Caenorhabditis elegans vulva is an excellent model to study how cell proliferation is regulated during animal development. During the first larval stage, six equivalent vulval precursor cells (VPCs) are born. These cells remain arrested in the G1 phase of the cell cycle until the beginning of the third larval stage, when their fates are specified and the three proximal VPCs proliferate to generate exactly 22 vulval cells. It is yet unknown how the exit of the 22 terminally differentiated vulval cells from the cell cycle is established and maintained throughout adulthood. We have previously found that the hox gene lin-39 is necessary to maintain the VPCs proliferating by regulating the expression of the two cell-cycle regulators cye-1 (cyclin E) and cdk-4 (Roiz et al. 2016). According to this model, the VPCs cease to divide as soon as the levels of LIN-39 drop below a certain threshold during the L4 stage. We have used the CRISPR-Cas9 system to generate a conditional knock-down allele of lin-39 that allows us to induce LIN-39 protein degradation at different stages during vulval cell proliferation. We observed that VulA and VulB cells lacking LIN-39 prematurely exit the cell cycle, undergoing only two instead of three rounds of cell division. Furthermore, using VPC-specific lin-39 over-expression we observed that cells expressing LIN-39 enter the cell cycle prematurely during the L2 stage, generating an excess of VPCs. These results indicate the LIN-39 is necessary and sufficient to promote VPC proliferation.

P-130 Physical and functional interaction between the SET1/COMPASS component CFP-1/CXXC and the Sin-3S/HDAC complex at promoter regions

Cecile Bedet[1], Flore Beurton[1], Matthieu Caron[1], David Cluet[1], Marion Herbette[1], Hélène Polvèche[1, 2], Przemyslaw Stempor[3], Yohann Couté[4], Alex Appert[3], Julie Ahringer[3], Francesca Palladino[1]
[1]Laboratory of Biology and Modeling of the Cell, UMR5239 CNRS Ecole Normale Supérieure de Lyon, INSERM U1210, UMS 3444 Biosciences Lyon Gerland, Université de Lyon, Lyon, France
[2]INSERM UMR 861, I-STEM, 28, Rue Henri Desbruères, 91100 Corbeil-Essonnes – France
[3]The Gurdon Institute and Department of Genetics, University of Cambridge, Cambridge U.K
[4]Grenoble Alpes, CEA, Inserm, BIG-BGE, 38000 Grenoble, France

The highly conserved CFP1/CXXC zinc finger protein targets SET1/COMPASS family complexes to promoter regions to implement methylation of histone H3 Ly4 (H3K4me), a mark that correlates with gene expression depending on the chromatin context. Whether CFP1 physically interacts with additional proteins on chromatin has not been investigated. Using a proteomics approach, we identify factors interacting with C. elegans CFP-1, and establish a link between CFP-1 and the Rpd3/Sin3 histone deacetylase (HDAC) small complex, which we identify for the first time. We show that CFP-1 and SIN-3 directly interact and, together with the SET1/COMPASS catalytic subunit SET-2, share common phenotypes and transcriptional targets. CFP-1 contributes to recruitment of both SIN-3 and the Sin3S subunit HDA-1 to H3K4me3 enriched promoters. Our results suggest that in addition to its well-characterized role as a central subunit of the SET1/COMPASS complex, CFP-1 interacts with the Rpd3/Sin3 HDAC small complex at promoter regions to influence gene expression in a developmental context. This has implications for the coordinate regulation of gene expression by chromatin associated complexes with distinct activities.

P-132 Decoding the rules for PATC-mediated prevention of gene silencing in C. elegans germline

Monika Priyadarshini, Christian Froekjaer-Jensen

Division of Biological and Environmental Science and Engineering King Abdullah University of Science and Technology Thuwal 23955-6900, Saudi Arabia

Non-coding DNA sequences, named periodic An/Tn clusters (PATCs) (1), can prevent germline-silencing of single-copy transgenes in repressive chromatin environments (2). Endogenous, germline-expressed genes contain PATCs when residing in repressive genomic regions where most transposons also reside; we speculate that PATCs license expression of endogenous genes to allow aggressive silencing of "foreign" DNA. We are interested in how PATCs prevent epigenetic silencing. Two PATC-rich genes, smu-1 and smu-2, are unusual because they are expressed in the germline from simple extrachromosomal arrays (3,4). Here, we demonstrate that PATC-rich transgenes are also expressed from arrays (>16 generations) and use simple arrays to test how PATCs prevent silencing. First, we have shown that a PATC-rich GFP fused to a neuron + germline promoter (snb-1) was expressed in both neurons and germline, in contrast to neuron-only expression using a standard GFP. Thus PATC-rich fluorophores more accurately reflect endogenous gene expression. Second, we have assessed whether PATCs are germline-specific enhancers. We paired a minimal promoter (pes-10) with a PATC-rich GFP and observed no obvious fluorescence. In contrast, the same GFP with two tissue-specific enhancers (ventral nerve cord and seam cells) located within introns showed the expected tissue-specific fluorescence. Thus PATCs do not appear to function as enhancers. Third, we are testing if PATCs confer transcriptional directionality, thereby preventing formation of dsRNA, by assessing the impact of reversing introns on germline fluorescence. Finally, we are testing if PATCs can perturb normal germline-specific gene regulation mediated by 3'UTRs (5), similar to how PATCs can prevent some piRNA-mediated silencing (6). Decoding the rules for PATC-mediated de-silencing will enable us to robustly engineer fluorophores and enzymes for easy germline activity and will guide our efforts to mechanistically understand how PATCs function. 1. Fire.., Genetics(2006). 2. Frøkjær-Jensen.., Cell (2016). 3. Spartz.., MCB (2004). 4. Spike.., MCB (2001). 5. Merritt.., Current Biology (2008). 6. Zhang..., Science (2018).

P-134 The C. elegans Aryl-Hydrocarbon Receptor (AHR-1) has evolutionary conserved functions and influences healthy ageing

Vanessa Brinkmann[1, 2], Alfonso Schiavi[1, 2], Anjumara Shaik[1], Lisa Tschage[1], Ralph Menzel[3], Natascia Ventura[1, 2]

[1]*IUF- Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany*

[2]*Heinrich Heine University Düsseldorf, Germany*

[3]*Humboldt-University Berlin, Germany*

Aging is concurrently shaped by genetic, environmental and nutritional interventions. In mammals the hazardous effects of many environmental toxicants are mediated by the aryl-hydrocarbon receptor (AhR), a highly conserved bHLH/PAS-containing transcription factor modulated through ligand–dependent (e.g., xenobiotics) or –independent (e.g., natural compounds) mechanisms. Specifically, AhR modulators can be divided into four classes: environmental, dietary, host-mediated, and microbial factors. We discovered that AhR promotes aging phenotypes across species,

(Eckers et al., 2016) and, more recently, indoles from bacteria were shown to extend C. elegans lifespan in an AhR-dependent manner (Sonowal et al., 2017). However, the classical AhR ligand, dioxin, does not bind the C. elegans AhR homolog AHR-1 (Butler et al., 2001) and no other potential modulators have been tested for their ability to influence C. elegans life traits through AHR-1. Therefore, in this study we investigated whether the C. elegans AHR-1 responds to any of the known classes of mammalian modulators. We specifically looked for gene expression and health-associated parameters in response to representatives of the four different classes. The environmental pollutant benzo(a)pyrene affected cyp expression and health independent of AHR-1. As host-mediated factor, we used UVB-produced FICZ, a highly potent AhR ligand in vivo. In response to UVB, both expression of detoxification genes and health were more affected in the ahr-1 mutants. Also, the dietary factor curcumin induced gene expression and increased health in an AHR-1-dependent manner. As microbial factors, we used different E. coli diets (HT115 vs. OP50), which we found to differentially impact on the health span of the ahr-1 C. elegans mutant. In conclusion, we showed that C. elegans is a good model for studying conserved AhR functions since it appears to be involved in the response to three out of four classes of mammalian AhR modulators, which we found to affect health span parameters.

P-136 Pro-longevity mitochondrial stress prevents C. elegans germline apoptosis through BRCA1/BARD1 tumor suppressor genes

Alfonso Schiavi[1, 5], Alessandro Torgovnick[4], Henok Kassahun[2], Anjumara Shaik[1], Silvia Maglioni[1], Shane Rea[6], Sebastian Honnen[3], Björn Schumacher[4], Hilde Nilsen[2], Natascia Ventura[1, 5]

[1]Leibniz Research Institute for Environmental Medicine (IUF), Düsseldorf, Germany

[2]Department of Clinical Molecular Biology, University of Oslo, and Akershus University, Norway

[3]Institute of Toxicology, Medical Faculty, Heinrich Heine University of Düsseldorf, Germany

[4]Institute for Genome Stability in Aging and Disease, Medical Faculty, CECAD Research Center, University of Cologne, Germany

[5]Institute for Clinical Chemistry and Laboratory Diagnostic, Medical Faculty, Heinrich Heine University of Düsseldorf, Germany

[6]University of Washington Seattle

In Caenorhabditis elegans, interventions and processes that extend lifespan are often identified in screens primarily designed for enhanced resistance to stress. Indeed, the efficacy of mechanisms that protect against different types of stressors declines with age and interventions that promote healthy aging are typically associated with increased resistance to stress. Paradoxically, reducing the activity of core biological processes such as mitochondrial metabolism or protein translation promotes animal lifespan extension and resistance to stress. In this study we set out to investigate whether the extended C. elegans lifespan elicited by partial reduction of mitochondrial functionality correlates with animals resistance to genotoxic stress and whether this is simply associated with or causally involved in their longevity. We found that reducing mitochondrial activity confers germline resistance to DNA-damage-induced cell-cycle arrest and apoptosis. Notably, this protective effect is not due to impaired apoptotic machinery but rather to a more efficient genomic maintenance apparatus. A small-scale RNAi screen revealed that the anti-apoptotic effect promoted by mitochondrial stress relies on the C. elegans homologs of the BRCA1/BARD1 tumor suppressor genes, brc-1/brd-1. On the other hand, lifespan upon reduced mitochondrial activity was still extended in the absence of brc-1 or brd-1 and was affected by genotoxic stress similar to that of wild-type animals. Thus, at least in animals with partial reduction of mitochondrial functionality, the mechanisms activated during development to safeguard the germline against genotoxic stress are uncoupled from those required for somatic fitness and animal longevity.

P-138 A mitochondrial isocitrate dehydrogenase prevents direct reprogramming of germ cells to neurons in C. elegans

Nida ul Fatima[1, 2], Ena Kolundzic[1], Anna Reid[1], Baris Tursun[1]

[1]*The Berlin Institute for Medical Systems Biology (BIMSB), Max Delbrück Centre (MDC) for Molecular Medicine in the Helmholtz Association*

[2]*National University of Sciences and Technology (NUST), Islamabad, Pakistan*

Direct reprogramming makes use of transcription factors (TFs) that induce the identity of specific cell types. These TFs often act in a context dependent manner, and are restricted in most cell types by inhibitory mechanisms that maintain cell fates. In order to identify these barriers in C. elegans we are using the zinc-finger TF CHE-1 that is required to induce the glutamatergic ASE neuron fate. Upon ectopic expression of CHE-1 and removal of barrier genes by RNAi, induction of the ASE neuronal fate marker can be seen in a variety of cell types. We identified a candidate barrier gene for reprogramming germ cells into neurons, the NAD+-dependent mitochondrial isocitrate dehydrogenase idha-1. Upon RNAi knockdown of idha-1 and ectopic expression of CHE-1, cells in the germline acquire neuron-like morphology and express a number of neuronal fate markers. Furthermore, upon ectopic expression of the Pitx family of homeodomain-containing TF UNC-30, which specifies the fate of GABAergic motor neurons, germ cells express a reporter for the GABAergic neuron fate upon knockdown of idha-1. Recent studies on mitochondria in the context of reprogramming and iPSCs, show that mitochondrial dynamics change during the process of differentiation. This suggests that disturbing mitochondrial function may feed back to chromatin thereby altering gene expression and allowing reprogramming. Interestingly, the idha-1 depletion-mediated reprogramming of germ cells to neurons is partially repressed in animals that lack the hypoxia-induced factor HIF-1. HIF-1 has been implicated in regulating iPSC reprogramming, a process that is triggered by changes in the level of the metabolite alpha-ketoglutarate. Importantly, alpha-ketoglutarate levels are regulated by isocitrate dehydrogenases such as IDHA-1. Moreover, alpha-ketoglutarate is known to act as a co-factor of histone demethylases8 and we are currently studying the underlying signaling mechanisms as well as the chromatin regulation involved during this cellular conversion.

P-142 mTOR complex 2 negatively regulates autophagy via Serum- and Glucocorticoid-inducible Kinase (SGK-1) independent of DAF-16/FoxO

Helena Aspernig[1], Wenjing Qi[1], Antje Thien[1], Ralf Baumeister[1, 2, 3]

[1]*Institute for Bioinformatics and Molecular Genetics (Faculty of Biology), University of Freiburg, D-79104 Freiburg, Germany*

[2]*ZBMZ Center for Biochemistry and Molecular Cell Research (Faculty of Medicine)*

[3]*Centre for Biological Signaling Studies (BIOSS)*

Autophagy is a highly conserved, degradative process that is crucial for the breakdown of macromolecules as well as for the removal of deleterious organelles. Autophagy thus represents a critical mechanism of cellular homeostasis and deregulation of autophagy has been linked to various types of human diseases like cancer, metabolic- and neurodegenerative disorders. The serine/threonine-protein kinase mechanistic target of rapamycin (mTOR/ C. elegans LET-363) is a highly conserved regulator of growth that functions in two distinct complexes. mTOR complex 1 is very well known to inhibit autophagy but the function of mTOR complex 2 in autophagy regulation is far less clearly defined. mTOR complex 2 has been previously proposed to inhibit autophagy in mouse muscle via activation of the AGC kinase Akt, which in turn phospho-inhibits the transcription factor FoxO3 to suppress the transcription of autophagy-related genes. We find that mTOR complex 2 inhibits autophagy in vivo in C. elegans. Interestingly, this function is mediated via serum- and glucocorticoid-inducible kinase (SGK-1), an AGC kinase that is known to be upregulated in various cancer types and has been linked to Akt-inhibitor resistance. In C. elegans, SGK-1 has been reported to act in a complex with AKT-1/-2 to phosphorylate DAF-16/FoxO transcription factor and to mediate certain aspects of DAF-2/insulin-signaling. Importantly, we find that SGK-1's function in autophagy regulation is independent of DAF 16/FoxO whereas AKT-1/-2 regulate autophagy in a DAF-16/FoxO-dependent manner. This suggests that SGK-1 and AKT-1/-2 regulate autophagy through distinct mechanisms. Our data establish mTOR

complex 2 as an important autophagy regulator in C.elegans and reveal a novel - FoxO independent - mechanism of mTOR complex 2 mediated autophagy regulation.

P-144 Role of KDM8/Jmjd-5 in preserving genome stability and germ line immortality

Nico Zaghet, Pier Giorgio Amendola, Anna Elisabetta Salcini

BRIC, University of Copenhagen

Modifications occurring on histone tails impact several DNA-based processes, including transcription, replication and DNA damage repair, by both modifying the interaction with the wrapping DNA and by creating binding sites for several chromatin factors. C. elegans germline is a great system to investigate the functional roles of chromatin factors and enzymes involved in histone post-translational modification. Mis-regulation of enzymes fine-tuning histone lysine methylation (lysine demethylases and transferases) has been implicated in germ cell protection after stress. Recent results indicate that methylation of histone tails in germ cells can also carry germ cell memory from one generation to the next one, thus protecting germ cell identity. Our lab identified JMJD-5 as a regulator of H3K36me2 required for genome stability after irradiation (Amendola et al, 2017). During our analyses, we also noticed that jmjd-5 mutants show progressive reduction of fertility, when mutant animals were grown for few generations at 25C, suggesting that JMJD-5, and most likely H3K36me level, are important for germ cell maintenance and fertility. To dissect the role of JMJD-5 on these processes, we are investigating the impact of jmjd-5 loss or inactivity on P-granules components and on transcriptional regulation, using immunofluorescence and RNA sequencing approaches, specifically in germ cells.

P-146 AFF-1-coated pseudoviruses as a novel tissue-specific delivery system can improve neuronal regeneration by fusion in *C. elegans*

Anna Meledin, Benjamin Podbilewicz

Department of Biology, Technion- Israel Institute of Technology, Haifa, Israel

Cell-cell fusion is fundamental for biological processes such as fertilization, organogenesis, and viral-host interactions. Among the FUSEXINS, a superfamily of structurally-related sexual, viral and somatic fusion proteins (fusogens), are the *Caenorhabditis elegans* EFF-1 and AFF-1. These fusogens mediate fusion of several cell types, and their expression pattern is known (1). Both EFF-1 and AFF-1 are bilateral; they are required on membranes of both cells for fusion, unlike the Vesicular Stomatitis Virus (VSV) glycoprotein G, which is unilateral. It has been shown that AFF-1-coated VSV, lacking its own glycoprotein G (VSVΔG-AFF-1) is able to fuse with Baby Hamster Kidney (BHK) cells expressing AFF-1 (2). Furthermore, VSV coated by G (VSVΔG-G) can infect living nematodes (3). Therefore, we asked whether VSVΔG-AFF-1 can serve as a tissue-specific delivery system in living *C. elegans* , infecting only EFF-1/AFF-1 expressing tissues. VSVΔG-AFF-1 was prepared in BHK cells, concentrated and injected into immobilized nematodes, followed by infection pattern analyses by confocal microscopy. We found that VSVΔG-AFF-1, but not VSVΔG-G, strongly infects the hyp7 hypodermal syncytium, known to express EFF-1. Moreover, while *aff-1* absence does not abrogate this infection, in *eff-1* null function mutants VSVΔG-AFF-1 cannot infect hypodermal cells. In addition, *eff-1* temperature sensitive (ts) worms, maintained at the permissive temperature produce wild-type-like infection, while at the restrictive temperature completely blocks hypodermal infection. Remarkably, transferring *eff-1(hy21ts)* animals from restrictive to permissive temperatures at the middle of development yields partially infected hypodermis. Hence, hypodermal cells specifically require EFF-1 expression for infection by VSVΔG-AFF-1. As AFF-1 was recently demonstrated to act cell-non autonomously to regenerate the injured PVD neuron by fusion (4), we asked whether we can use VSVΔG-AFF-1 to externally supply AFF-1 into animals with injured PVD. Our preliminary data suggests that infection by VSVΔG-AFF-1 improves dendrite regeneration in aged worms. (1) Hernandez and Podbilewicz (2017) (2) Avinoam, et al. (2011) (3) Gammon, et al. (2017) (4) Oren-Suissa, et al. (2017)

P-148 Genetic Control of the Maintenance of AIA Cell Identity

Josh Saul, Takashi Hirose, Bob Horvitz

HHMI, Dept. Biology, MIT, Cambridge, MA 02139

As development progresses in multicellular organisms, the developmental potential of an individual cell becomes increasingly restricted until a final differentiated cellular identity is adopted. Work in recent years has indicated that a cell's differentiated identity can be unstable, and the need for factors that maintain this differentiated identity is apparent though poorly understood. Here I present the characterization of a gene, ctbp-1, that might act to maintain the identities of one or more cell types in C. elegans. ctbp-1 encodes the worm homolog of the C-terminal Binding Protein (CtBP) family of proteins, shown in mice and Drosophila to function as transcriptional corepressors that act during development. ctbp-1 mutant adult worms show defects in the gene expression, cellular morphology and cellular function of the embryonically-born AIA neurons. By contrast, ctbp-1 L1 worms do not display any of these defects. We hypothesize that ctbp-1 worms properly establish the AIA cellular identity but fail to maintain this identity as the worm ages. To understand how ctbp-1 acts to regulate AIA cellular identity, we have performed a genetic suppressor screen looking for reversion of AIA gene misexpression observed in ctbp-1 animals. From this screen we identified rpm-1, which encodes an E3-ubiquitin ligase previously studied for its role in synaptogenesis and axon termination. How ctbp-1 and rpm-1, two seemingly disparate factors, converge to maintain the integrity of a cell's identity is unknown. We are seeking additional genes that interact with ctbp-1 to help clarify the functional relationship between ctbp-1 and rpm-1 and elucidate the mechanism by which AIA cell identity is maintained. We hope that by understanding the maintenance of cellular identity in C. elegans we will gain insights into instances in which a failure of cell-identity maintenance can lead to disease, as in the reprogramming of cellular identity during cancer initiation.

P-150 The activity of unc-1/stomatin-like protein in the nervous system modulates protein homeostasis in C. elegans.

ANA PILAR GÓMEZ ESCRIBANO[1], JOSE BONO YAGÜE[1], IRENE REAL ARÉVALO[1], QIUYI CHENG ZANG[1], JOSE BLANCA[2], MARIA DOLORES SEQUEDO PÉREZ[1, 4], JOAQUÍN CAÑIZARES[2], JUAN BURGUERA[3], CARMEN PEIRÓ[3], JOSÉ MARIA MILLÁN SALVADOR[1, 4], RAFAEL VÁZQUEZ MANRIQUE[1, 4]

[1]*Laboratory of Molecular, Cellular and Genomic Biomedicine, Instituto de Investigación Sanitaria La Fe, Valencia, Spain*

[2]*Centro de Conservación y Mejora de la Agrodiversidad Valenciana (UPV-COMAV), Valencia, Spain*

[3]*Servicio de Neurología, Hospital Universitario y Politécnico La Fe*

[4]*Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Valencia, Spain*

Toxicity of proteins containing polyglutamines (polyQs) is one of the main causes of diseases produced by abnormal CAG expansions, like for example Huntington's disease (HD). Mutant huntingtin (mHtt) is very prone to aggregation, which is believed to cause aggregation of other molecules, and contributes to the toxicity in patients of HD. Despite HD is a monogenic disease, there may be genes that modify the dynamics of mHtt aggregation and clearance, and therefore may modulate the progression of the disease in HD patients. To look for modifier genes that modulate the dynamics of aggregation of polyQ-containing proteins, we performed an EMS screen on AM141 worms. This strain expresses 40 glutamines (40Q) fused in frame with YFP in muscle cells. 40Q::YFP aggregates in an age-dependent fashion. We isolated an enhancer of aggregation. We identified, using NGS, the responsible mutation, probably a null allele (vlt10) in the unc-1/Stomatin like protein 3 gene. To verify the role of unc-1, we have introduced different unc-1 alleles within AM141 which confirmed that unc-1 is a modulator of aggregation. Here we show that rescuing unc-1 in the nervous system modifies aggregation of 40Q::YFP in muscle cells, suggesting that unc-1 act non-cell autonomously. To look for potential mechanisms of protein aggregation modulation by unc-1, we performed genetic interaction studies. Among genes and pathways tested, we observed that the unc-1 phenotype is rescued by loss of function of ssu-1, a gene encoding a sulfotransferase enzyme which expression is restricted to the ASJ neuron. It is believed that SSU-1 adds sulphur to hormones produced by ASJ, to facilitate distribution among tissues. Since ablating ssu-1 rescues unc-1(vlt10) animals, we hypothesises that unc-1 animals show a defect of signalling of some hormones, which in turns alters protein homeostasis in other tissues. We have analysed the insulin and steroid signalling pathways to further explore this mechanism.

P-152 Gene expression profiles and alpha-synuclein toxicity varys in a C. elegans model of PD with variable natural genetic backgrounds

Yiru Wang[1, 2], Basten Snoek[1], Mark Sterken[1], Joost Riksen[1], Jana Stastna[2], Yu Nie[2], Jan Kammenga[1], Simon Harvey[2]

[1]Laboratory of Nematology, Wageningen University, Wageningen, The Netherlands

[2]Biomolecular Research Group, School of Human & Life Sciences, Canterbury Christ Church University, Kent, United Kingdom

Neurodegenerative diseases (NGDs), such as Alzheimer's diseases (AD) and Parkinson's diseases (PD), are characterized by progressive degeneration in the human nervous system. The nematode C. elegans is an excellent model in which to study NGDs due to the high level of conservation of gene functions compared to humans. However, C. elegans research largely relies on a single worm genotype – the canonical N2 strain – limiting the ability to explore how naturally varying alleles alter pathological mechanisms in NGDs. In order to identify how genetic variation acts on NGDs, we analyzed transgenic animals that express aggregating human proteins associated with molecular pathogenic progression of NGDs in five genetic backgrounds. Here, starting with the original transgenic strain expressing the human synaptic protein alpha-synuclein in an N2 genetic background, we have introgressed the PD transgene (unc-54::α-Syn::YFP) into four different wild type genetic backgrounds. Analysis of these new transgenic introgressed lines indicates that transgene effects vary greatly depending on the genetic background. To understand the genetic bases of these phenotypic differences, we measured various aspects of the life history, and investigated gene expression differences by microarray. These analyses identified genes that are up- and down-regulated in all genotypes and genes that expressed at a specific stage to particular genetic backgrounds. Functional enrichment links these genes to the aggregation of alpha-synuclein, which is causative of PD, to the associated developmental arrest, metabolic, and cellular repair mechanisms. There is variation between isolates during the investigation of worm healthspan, including the genotype-specific on lifespan, developmental delay, mobility deficits, pumping arrest and variable aggregations. Our studies employ C. elegans PD models with different genetic backgrounds to identify their alterations on alpha-synuclein aggregates and the core alterations in global gene expression associated with the toxicity of alpha-synuclein aggregation. This is relevant to our understanding of the diseases of protein misfolding.

P-154 A pseudogene going astray: Is the transdifferentiation inhibitor F55A3.7 a novel non-coding RNA?

Andreas Ofenbauer, Ena Kolundžić, Alexander Gosdschan, Baris Tursun

Berlin Institute for Medical Systems Biology (BIMSB) at Max Delbrück Center (MDC)

We recently identified the chromatin regulator FACT (Facilitates Chromatin Transcription) as a cellular reprogramming barrier in C. elegans and H. sapiens. FACT is a heterodimer consisting of the histone-binding protein SPT-16 and either HMG-3 or HMG-4 (SSRP1 in H. sapiens) in C. elegans Ectopic overexpression of the ASE neuron fate-inducing Zn-finger transcription factor (TF) CHE-1 in FACT depleted C. elegans, enables germ cells to express the reporter gcy-5p::gfp, which is specific for the glutamatergic ASER neuron. These converted germ cells also display morphological changes, including axo-dendritic like projections and expression of pan-neuronal markers such as rab-3 or unc-119. We report now the identification of a previously uncharacterized pseudogene, F55A3.7, which shares more than 90% sequence homology with the FACT subunit-encoding gene spt-16. Strikingly, the ok1829 deletion allele of F55A3.7 allows germ cell conversion upon broad CHE-1 overexpression, mimicking the knock-down effect of other FACT members. We could detect the transcript of F55A3.7 using RT-PCR, but no protein upon a 3xFLAG CRISPR knock-in, confirming that F55A3.7 is expressed. F55A3.7 acts in trans, as we can rescue the mutant phenotype by driving the WT pseudogene from an extrachromosomal array (several lines). We performed qRT-PCR of dissected gonads to check for germline-specific transcript levels of the mother gene spt-16 in the ok1829 mutant background, as reduced levels of spt-16 would be the simplest explanation for the germline conversion phenotype. However, germ-line specific transcript levels of spt-16 (as well as the other FACT subunits) were unaltered, suggesting that the putative non-coding RNA F55A3.7 acts through other gene regulatory pathways. To reveal how the putative non-coding RNA F55A3.7 safeguards the germ cell identity, we performed germline-specific RNA-seq and ATAC-seq, which will be reported at the meeting.

P-156 Sleep counteracts aging phenotypes to promote survival of starvation

Henrik Bringmann, Florentin Masurat, Yin Wu, Jasmin Preis

Max Planck Institute for Biophysical Chemistry Goettingen

Sleep is ancient and fulfills higher brain functions such as memory consolidation as well as basic vital processes. Little is known about how sleep emerged in evolution and what essential functions it was selected for. Here we investigated sleep in Caenorhabditis elegans across different life stages and conditions to find out why sleep in a simple animal becomes essential for survival. Food conditions control sleep, with extended starvation being a major trigger. Conserved nutrient-sensing longevity pathways, AMP kinase and FoxO, act in parallel to induce sleep during starvation through depolarization of the single sleep-active neuron RIS. While sleep does not appear to be essential for a normal adult lifespan, it is crucial for survival of larval arrest triggered by the absence of food, a function that it likely was selected for in evolution. Rather than merely saving energy for later use, sleep acts by slowing the progression of aging phenotypes. Thus, sleep is a protective anti-aging program that is induced by nutrient-sensing longevity pathways to survive starvation and developmental arrest.

P-158 Genetic analysis of a sas-6 mutant reveals mitotic and meiotic differences in centriole duplication and stability

Kevin O'Connell[1], Nicole DeVaul[1], Gunar Fabig[2], Kevin O'Connell[2]

[1]*National Institutes of Health-USA Bethesda, MD 20892*

[2]*Technische Universität Dresden, Germany*

Centrioles are nine-fold symmetric barrel-shaped organelles that exist as mother-daughter pairs and participate in the assembly of bipolar spindles and the formation of cilia and flagella. During S phase of each cell cycle, centrioles are precisely duplicated through a process that involves the formation of a new daughter next to each preexisting mother centriole. In C. elegans, as in other species, five core centriole assembly factors are required for centriole duplication and these same factors function in different cell types, suggesting that a similar assembly mechanism operates in all tissues. SAS-6 is one of the core assembly factors and plays a critical role in centriole assembly by forming a central scaffold around which the daughter is built. Here we describe a missense (D9V) mutation in C. elegans SAS-6 that strongly affects the formation and/or stability of centrioles in the meiotic portion of the male germ line but has little effect on centrioles in the mitotic environment of the early embryo. Examination of mutant male germ lines indicates that both the incorporation of SAS-6(D9V) into daughter centrioles and centriole assembly occur during pre-meiotic centriole duplication. However, during meiosis daughter centrioles fail to form and SAS-6(D9V) is gradually lost from most of the mother centrioles. As a result, sperm inherit a single structurally-disorganized centriole that further deteriorates after it is transferred to an embryo at fertilization. Despite a strong loss of structural integrity, these centrioles organize normal-size centrosomes and duplicate at high rates during the embryonic divisions. Thus, the D9V mutation preferentially affects centriole stability and duplication during meiotic divisions. Further genetic and cytological analysis of this mutant will allow us to identify tissue-specific mechanisms that ensure the structural stability and reproductive capacity of centrioles during development.

P-160 Modeling rare monogenic human diseases in C. elegans

Andy Golden, Ben Nebenfuehr, Carina Graham, Tyler Hansen, Peter Kropp, Isabella Zafra-Martinez

LBG/NIDDK/NIH

There are currently ~7,000 identified rare diseases and this list continues to grow. Eighty percent of these diseases are thought to be genetic in origin. For many of these human diseases, gene orthologs can be readily identified in C. elegans. My group models rare human monogenic diseases in C. elegans using CRISPR/Cas9 genome editing. We generated deletions of these disease genes to identify and characterize the null phenotypes as well as made patient-specific alleles in the C. elegans orthologs to determine whether they also disrupt function. We are investigating two genes known to cause cardiac arrhythmias in humans (kqt-3 and egl-19), another gene responsible for NGLY1 deficiency (png-1), another for a lipodystrophy disease (seip-1), and another for Multiple Mitochondrial Dysfunction

Syndrome 1 (lpd-8). We have identified phenotypes for each and hope to characterize each of these disease models at the cellular and molecular level. We plan to carry out suppressor screens with each of these models to identify extragenic mutations. The identification of such suppressors may lead to novel therapeutic targets for those treating these diseases. We will present data showing our progress with each of these projects. We are also collaborating with the Undiagnosed Disease Program and WormBase to highlight newly identified patient missense mutations that are conserved between humans and C. elegans to encourage more research on these rare disease genes.

P-164 Unequal distribution of centrosomal lin-5 mRNA during asymmetric division of C. elegans embryos

Alexandra Bezler[1], Zoltán Spiró[1, 2], Pierre Gönczy[1]

[1]Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland

[2]present address: Institute of Science and Technology Austria, Am Campus 1, 3400 Klosterneuburg, Austria

Asymmetric cell division is essential for generating cell diversity during development. The C. elegans zygote allows to investigate mechanisms of asymmetric spindle positioning in a developing organism. The mechanisms underlying force generation during spindle positioning are thought to act strictly at the protein level. We report that the mRNA encoding the force generation component LIN-5 is enriched around centrosomes in a microtubule and dynein-dependent manner in one-cell C. elegans embryos; this intriguing localization is conserved in C. briggsae. Centrosomal localization of lin-5 mRNA becomes asymmetric during mitosis, with stronger enrichment on the anterior centrosome. We show that the lin-5 coding sequence is sufficient for centrosomal mRNA enrichment. In addition, lin-5 mRNA is mislocalized by a 9-nucleotide insertion in the coding sequence. Importantly, morpholino-mediated inhibition of lin-5 translation in the newly fertilized embryo impairs posterior pulling forces, indicating that de novo LIN-5 protein production is critical for proper asymmetric cell division.

P-166 A Zona Pellucida domain protein and Patched-related protein promote apical ECM assembly.

Jennifer Cohen, Rachel Forman-Rubinsky, Meera Sundaram

University of Pennsylvania Perelman School of Medicine, Department of Genetics.

A lipid-, sugar-, and glycoprotein-rich apical extracellular matrix (aECM) shapes and protects apical surfaces, like those of the epidermis and tube lumens. For example, a glycocalyx ("sweet husk") lines capillaries in the vascular system. Although damage to aECMs may contribute to epithelial integrity defects, how aECMs assemble remains poorly understood. To identify genes that promote tube integrity, our lab performed forward genetic screening in C. elegans. We identified a set of apically secreted proteins that are required to maintain the excretory system's middle tube, the duct cell, as it elongates during embryogenesis. These proteins also shape epidermal ridges (alae) and maintain embryonic epidermal integrity. One such protein is LET-653, which contains a Zona Pellucida (ZP) domain. ZP protein dysfunction is associated with human diseases, including the microvascular disease hereditary hemorrhagic telangiecstasia (HHT), but how ZP proteins assemble and traffic in the aECM is unclear. ZP domains consist of two parts, ZP-N and ZP-C. In vitro studies suggest that ZP-N domains polymerize to form fibrils, while ZP-C domains regulate ZP-N polymerization and/or bind specific partners to alter signaling. However, we found that the LET-653 ZP-C domain is sufficient to rescue let-653 mutants and to form a stable (though transient) aECM layer; we are now testing if it can polymerize on its own. Many ZP proteins, including LET-653, are cleaved at their C-termini in a reaction that may promote ZP-N polymerization. Although LET-653 functions via its ZP-C domain, we found that LET-653's cleavage site is required for LET-653 function and aECM layer localization. To find partners that recruit LET-653 to its aECM layer, we performed an RNAi screen that identified the patched-related gene ptr-4. Patched-related genes encode multi-pass transmembrane proteins with poorly described function and intracellular localization. Continuing work will test whether PTR-4 anchors a LET-653 aECM complex or promotes LET-653 trafficking/cleavage.

P-168 TORC1 hyperactivation effects in Caenorhabditis elegans' nervous system

Aikaterini Stratigi[1, 2], Patrick Laurent[1, 2]

[1]*Université Libre de Bruxelles, Laboratory of Neurophysiology, Faculty of Medicine, Belgium*

[2]*Université Libre de Bruxelles, ULB Institute for Neuroscience (UNI), Belgium*

The mTORC1 (mammalian target of rapamycin) signaling pathway is involved in multiple major cellular functions, including protein synthesis, cell growth and proliferation. Mutations causing the hyperactivation of mTORC1 signaling are a common occurrence of human epilepsies, offering new therapeutic targets. In the mammalian nervous system, mTORC1 hyperactivity affects neuronal development, neuronal morphology, neuronal excitability and synaptic plasticity. Despite the extensive study of mTORC1 pathway during the last decade in multiple cell types, how its hyperactivity modifies the neuronal function remain unclear. We explore the effects of mTORC1 hyperactivity using the genetic toolbox of C. elegans. Few is known about the function of TORC1 in C. elegans nervous system. Our first step was to investigate the phenotypes of mutants leading to TORC1 hyperactivation. We focused on potential behavioral phenotypes as well as on the morphology of specific neurons. Our goals are the characterization of mTORC1 effects on neuronal function and the potential mTORC1 targets in the neurons, and how the environment might influence the phenotypes identified.

P-172 A forward genetic screen to identify temperature-sensitive, early cell division mutants

Thalia Padilla, Nick Jackson, Bruce Bowerman

Institute of Molecular Biology, University of Oregon, Eugene, OR.

Cell division plays an important role in the development of an organism, and we are interested in understanding the basic requirements for proper early cell division in Caenorhabditis elegans. Upon fertilization and completion of meiosis, the embryo embarks on its first mitotic division. The sperm and oocyte-derived pronuclei, located at opposite ends of the embryo, migrate towards each other to meet and fuse, initiating mitosis. The mitotic bipolar spindle forms and begins to rock, and chromosomes congress along the metaphase plate and are pulled to opposite poles during anaphase. After cytokinesis, the two daughter cells (AB and P1) have identical complements of DNA. The Bowerman Lab has a collection of ~1000 temperature-sensitive, embryonic lethal (TS-EL) mutants, and we have screened the final 143 TS-EL mutants in the collection for early cell division defects using time-lapse Narmarski microscopy. To date, we have identified 21 mutants with a variety of partially or fully penetrant early cell division defects. Five mutants, or1144, or1305, or1379, or1711 and or1900 have penetrant defects including extra pronuclei, chromosome segregation abnormalities, and cytokinesis failure. To further characterize these mutants, we are performing genetic tests to give priority to loss-of-function, single-mutation mutants. Lastly, we use SNP mapping and whole genome sequencing to locate and identify the mutated genes. We have cloned 2 mutants, or1305 and or1379, representing two genes, drp-1 and atl-1, respectively. drp-1 encodes a dynamin related protein required for mitochondrial division, and atl-1 encodes a protein with a PI-3 kinase-like domain and is required for normal chromosome segregation. We anticipate that these and other mutants we characterize will provide a resource that enhances our efforts to better understand cell division.

P-174 Modulation of FMRF like neuropeptides genes (flps) transcription by neuroendocrine signalling in C. elegans

J. Ramiro Lorenzo, Aikaterini Stratigi, Patrick Laurent

Université Libre de Bruxelles, UNI, Laboratory of Neurophysiology, Belgium

Neurons and endocrine cells display a regulated secretory pathway in which neuropeptides and peptide hormones are loaded in large dense-core vesicles to be secreted at the cell surface. Dense core vesicles require de novo biogenesis to replenish the stores, meaning that neurons need to produce more neuropeptides in response to an increases regulated secretory activity. Our laboratory reported a coupling between neuronal activity and transcriptional activity of several neuropeptide genes in C. elegans, including flp-5, flp-11, flp-17, flp-21. More recently we observed the same effect for flp-10 and flp-19. In this context, to get insight in how the neurosecretion influences the expression of flps, we studied the promoter activity of flps in different mutant backgrounds using GFP

as read out. Particularly, we test mutants with impaired or enhanced neurotransmission (unc-64, tom-1, and others) and observed significant changes in flp-11 and flp-10 promoter activities. Furthermore, to test how much of this effect is due to peptidergic modulation, we tested mutants with altered neuropeptide processing activity: pamn-1 and egl-21. Remarkably, these mutations have a great impact in flp-11 and flp-10 promoter activities suggesting that neuroendocrine signalling strongly modulates flps expression. To address the origin of this neuroendocrine signalling we are currently rescuing egl-21 in different tissues. Here we discuss our current results.

P-176 IDENTIFICATION OF LOW-DENSITY LIPOPROTEIN RECEPTOR-RELATED 8 INVOLVEMENT IN ALZHEIMER'S DISEASE

Carla Bertapelle[1, 2], Alessandro Medoro[1], Donatella Mignogna[1], Claudio Russo[1], Elia Di Schiavi[2]
[1]*Department of Medicine and Health Sciences, University of Molise, Campobasso, Italy*
[2]*Institute of Bioscience and BioResources, CNR, Naples, Italy*

Alzheimer's disease (AD) is the most common neurodegenerative disorder and its neuropathological hallmarks include the extracellular deposits of β-amyloid (Aβ) plaques and intraneuronal tangles, resulted from Tau hyperphosphorylation and aggregation, contributing both to the loss of synapse function and neuronal death. The major genetic risk factor in AD is represented by ApolipoproteinE (ApoE), ligand of the Low-Density Lipoprotein Receptors-Related-(LRPs) family. Among these receptors, LRP8 is expressed in neurons and is involved in memory processes, neuronal migration and proliferation. Moreover, its processing is regulated by γ-secretase cleavage in a similar way to the Amyloid Precursor Protein (APP), which after cleavage by γ-secretase produces the Aβ peptide. Considering the few information about LRPs involvement in AD, we propose a C.elegans model to study in vivo the correlation among LRP8, APP and Tau. Several C.elegans models have been generated to investigate APP processing, Aβ aggregation and Tau hyperphosphorylation. These models have shown neurodegenerative defects progressively increasing with aging, such as neuronal abnormalities (inclusions and varicosities in cell body), axonal degeneration (bulges, dilatation and collapse of axonal membrane) and altered neurotransmission, resulting in the deterioration of a variety of animal behaviors. To investigate the impact of LRP8 expression and processing on C.elegans nervous system function, we generated transgenic lines overexpressing the human LRP8 protein in all neurons. The analysis of these lines indicates that LRP8 affects the health, the fertility and the locomotion of the animals in a similar way as observed in other C.elegans AD models. Moreover, we inhibited the γ-secretase activity using both a pharmacological and a genetic approach, and we showed the sel-12 (γ-secretase ortholog) involvement in LRP8 processing. We will use this model to test our hypothesis that, during AD, LRP8 processing could affect APP processing and signaling (and vice versa) and Tau hyperphosporylation and aggregation.

P-178 The LSL-1 zinc-finger protein promotes meiotic prophase progression

David Rodriguez Crespo, Chantal Wicky
Department of Biology, University of Fribourg, Chemin du Musée 10, 1700 Fribourg, Switzerland

Chromatin and transcription factors are essential to establish the appropriate gene transcription profile during development. Among the chromatin factors that play a role in development, the chromatin remodeler Mi2 is known to regulate stem cell proliferation, differentiation and cell fate maintenance. Previous studies from our lab showed that the C. elegans Mi2 homolog, LET-418/Mi2, together with other chromatin factors, maintains proper germ cell identity, promotes post-embryonic development and represses ectopic expression of germline genes in the soma. In a genome-wide RNAi screen for suppressors of let-418 associated defects, we identified LSL-1, a zinc-finger protein, whose function is unknown. To gain further insights into the function of LSL-1 and to know how it could interact with LET-418 to promote development, we initiated a functional study. We found that lsl-1 expression is first observed at the birth of the primordial germ cells Z2 and Z3 and is maintained in proliferating and meiotic germ cells during larval stages and adulthood. Furthermore, we observed that LET-418 is required for its proper localization. Worms lacking lsl-1 activity show a defective progression of germ cells through meiotic prophase. Number of germline nuclei at the so-called transition zone, where chromosomes are clustered towards one side of the nucleus and initiate homologous pairing is higher. This defect is partially dependent on LET-418/Mi2 activity

suggesting that both proteins could play role in shaping chromatin during pairing and synapsis of the chromosomes for proper meiotic prophase progression. We are currently investigating genetically and molecularly how LSL-1 and LET-418/Mi2 are interacting with the pairing and synapsis machinery and where they are binding to the meiotic chromosomes to ensure the production of functional gametes

P-180 A C. elegans model for the histone H3.3K27M cancer driver mutation

Kamila Delaney, Maude Strobino, Joanna M. Wenda, Florian A. Steiner

Department of Molecular Biology and Institute for Genetics and Genomics in Geneva, University of Geneva, 1211 Geneva, Switzerland

Substitution of lysine 27 with methionine in histone H3 or H3.3 is a recently discovered driver mutation of pediatric high-grade gliomas. Tumor cells carrying the mutation show a dramatic decrease in H3K27me3 levels, presumably due to the physical inhibition of PRC2 methyltransferase activity by H3.3K27M. However, small regions of the genome retain the mark, which cannot be explained by global PRC2 inhibition. We generated a C. elegans model for the H3.3K27M oncohistone. H3.3K27M mutation results in germ line replication defects and endomitosis of oocytes. Moreover, H3K27me3 is not globally depleted, but redistributed from autosomes to the X chromosome, where low levels of H3.3K27M incorporation allow H3K27me3 accumulation. Induced increase in incorporation of H3.3K27M results in a local reduction of H3K27me3, indicating that PRC2 inhibition by H3K27M acts in cis. However, high levels of preexisting H3K27me3 can protect against loss of the mark. The redistribution of H3K27me3 genome wide leads to misregulation of hundreds of genes and results in severe replication defects, reflecting characteristics of tumor cells.

P-182 Natural variation in the genetic architecture of a germ stem cell niche

Sarah Fausett, Christian Braendle

Université Côte d'Azur, CNRS, Inserm, iBV

A fundamental goal of evolutionary biology is to understand the genetic and developmental mechanisms leading the great diversity of organismal life histories. Comparison among distant taxa cannot easily address this issue due to high complexity; however, it is possible to investigate genetic architecture underlying life history phenotypes within species. Here we show that C. elegans wild isolates from around the globe display extensive size variation of the germline mitotic zone (MZ), indicative of variation in the activity of the germ stem cell (GSC) niche. To characterize the molecular genetic differences explaining such variation in MZ size, we focus on two C. elegans isolates with strong differences in total germ cell number and MZ size. We constructed a panel of 144 SNP-genotyped F2 recombinant inbred lines (RILs) derived from the parental cross between these two isolates. Phenotyping of ~70 RILs and subsequent quantitative trait locus (QTL) analysis yielded a large effect QTL on chromosome II (~7.25 Mb) that acts additively with a QTL on chromosome V (~2.6Mb). We are currently aiming to identify the causal variant(s) in the chromosome II QTL through generation of near-isogenic lines and further fine-mapping. The results of these studies not only illuminate the genomic features underlying an important life history trait, but will lead to the identification of specific molecular changes that have given rise to natural variation in a stem cell niche.

P-184 Analysis of inter-individual transcriptional variability of stress response genes in C. elegans by high-throughput qRT-PCR in single worms

Laetitia Chauve[1], Catalina Vallejos[2, 3, 4, 5], Janna Hastings[1], John Marioni[4, 6], Olivia Casanueva[1]

[1]Epigenetics Department, Babraham Institute, Cambridge CB223AT, United Kingdom.

[2]The Alan Turing Institute, British Library, 96 Euston Road, London NW1 2DB, United Kingdom

[3]Department of Statistical Science, University College London, 1-19 Torrington place, London WC1E 7HB, United Kingdom

[4]EMBL European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD, United Kingdom

[5]MRC Biostatistics Unit, Cambridge Institute of Public Health, Forvie Site, Cambrige Biomedical Campus, Cambridge CB2

0SR, United Kingdom
[6]Cancer Research UK Cambridge Institute, University of Cambridge, Li Ka Shing Centre, Robinson Way, Cambridge CB2 0RE, United Kingdom
Enzymes with chaperoning activity are essential to maintain protein-folding homeostasis (proteostasis). A reduction in proteostasis is associated with the loss of buffering against environmental stress and the increased penetrance and expressivity of temperature sensitive mutations (Gidalevitz et al., 2006; Casanueva et al., 2011). This buffering capacity is lost very early during the ageing process in the nematode C. elegans (Ben Zvi and Morimoto, 2009; Labbadia and Morimoto, 2015). Despite the importance of chaperones for so many essential processes, they are highly variable across stress-exposed worms. Inter-individual variability has consequences because its single-worm expression is predictive of mutation penetrance, lifespan and stress resistance (Rea et al., 2005; Casanueva et al., 2011). Much less is known about the variability of stress genes at basal levels. To monitor inter individual variability for multiple transcripts simultaneously, we have developed a method to quantify biological variance and co-variance -a good metrics for gene co-regulation by common upstream regulators. Ultimately, we want to build in-vivo reporters to study the phenotypic consequences of highly variable genes to identify new predictors of health and lifespan. To monitor inter-individual variability in gene expression, we have established a high-throughput quantitative real-time PCR assay on single worms using nano-fluidic technology. We have also developed an integrative Bayesian statistical method to estimate biological variability from PCR datasets, while accounting for technical variability. This technique enables us to identify subsets of highly variable stress response genes. To determine whether specific tissues drive the observed inter-individual variability, we are currently investigated expression pattern of variable gene in individual worms, using single molecule RNA Fluorescent In Situ Hybridization. Our aim is to determine the nature of inter-individual variability in gene expression and to exploit inter-individual variability in gene expression as a method to study gene network architecture in a metazoan model organism.

P-186 Investigating the molecular mechanisms of SET-24 in maintaining germline immortality

Giulia Furlan[1, 2], Lise Frézal[4], Ahmet Can Berkyurek[1, 2], Alper Akay[1, 2], Marie-Anne Felix[4], Eric Miska[1, 2, 3]
[1]The Gurdon Institute, University of Cambridge, UK
[2]The Department of Genetics, University of Cambridge, UK
[3]Wellcome Sanger Institute, Cambridge, UK
[4]Institut de Biologie de l'Ecole Normale Supérieure, CNRS, INSERM, ENS, Paris Sciences et Lettres, Paris, France
Germline proliferation and maintenance are essential processes in development and are required to ensure the proper transmission of genetic and epigenetic information to the offspring. In the C. elegans germline, Argonaute-mediated small RNA pathways direct genome surveillance to ensure proper inheritance and maintain fertility. Two of these are the piRNA pathway, which guides the repression of parasitic genomic elements, and the germline nuclear RNAi pathway, which mediates environmentally-induced transgenerational gene silencing. Defects in these pathways lead to sterility, with mutant individuals exhibiting a mortal germline (Mrt) phenotype, in which a progressive decline in fertility accumulates across generations, ultimately resulting in complete sterility. Importantly, this phenotype is enhanced by environmental stress conditions such as high temperatures. Interestingly, the heat-dependent Mrt phenotype typical of small RNA pathway mutants can be found in some C. elegans wild isolates. Recent collaborative work by the Felix and Miska laboratories has identified the set-24 gene as the major causal locus of the strong Mrt phenotype observed in specific wild strains. Set-24 encodes a germline-specific protein with an N-terminal SET domain. set-24 mutant animals reach sterility in less than 10 generations when chronically exposed to restrictive (25C) temperatures. Current work is aimed at elucidating the molecular contribution of SET-24 to small RNA pathways. Preliminary results indicate that SET-24 is dispensable for piRNA activity and global H3K9me3 deposition. Ongoing experiments are focusing on testing set-24 contribution to the nuclear RNAi pathway, and at identifying SET-24 interactors and antagonists.

P-188 Investigating the mechanism of chromatin domain formation

Garima Sharma, chiara cerrato, Ni Huang, Julie Ahringer

The Gurdon Institute and University of Cambridge, Tennis Court Road, Cambridge UK
C. elegans autosomes are partitioned into alternating domains of differently marked chromatin. “Active” domains are enriched for H3K36me3 and other modifications associated with gene activity, and they contain genes that have broad expression across development and cell types. “Regulated” domains are marked by H3K27me3 and contain genes with developmentally or conditionally regulated expression. In Drosophila, similar chromatin domains are observed, marked by low or high levels of H3K27me2 instead of H3K27me3, indicating that this chromatin domain structure is conserved. H3K36me3 is deposited co-transcriptionally by MET-1, while maternally provided, germline-specific MES-4, maintains patterns of germ line H3K36me3 marking in early embryos. H3K27me3 is deposited by the PRC2 complex (MES-2, MES-3, MES-6) in the germline and in early embryos but an unknown activity generates H3K27me3 somatically in late embryos and later in development. In mes-4(RNAi) embryos, H3K27me3 spreads into active domain genes (which are germ line expressed), indicating that MES-4 plays a role in domain formation. We are studying how active and regulated domains are formed and the function of domains in gene expression. To investigate the potential role of PRC2, we mapped H3K36me3 in mes-2 (m-z-) embryos. We found that H3K36me3 marking of active domains is normal, which is consistent with the classical role of PRC2 in maintaining gene repression and supports the idea that specification of active domains may be a primary driver of the domain pattern. To try to identify the somatic activity that generates H3K27me3, we conducted a screen of 34 mutants/RNAi of genes encoding proteins with a SET methyltransferase domain, but we found that all single mutants still generated somatic H3K27me3. We are testing combinations of mutants in case of redundancy. We are currently testing potential roles of histone variants, other histone modifications, and chromatin proteins in domain formation.

P-190 Evolution of QR neuroblast migration and mig-1 regulation in C. elegans and other nematodes.

Clement Dubois, Marie-Anne Felix

Institut de biologie de l’Ecole normale supérieure (IBENS), Ecole normale supérieure, CNRS, INSERM, PSL Université Paris 75005 Paris, France.

In the first stage larva of Caenorhabditis elegans, the QR neuroblast migrates anteriorly, while undergoing three rounds of division. The two daughter cells of QR.pa, QR.paa and QR.pap (henceafter called QR.pax) acquire a neuronal fate and their final position can be scored by Nomarski microscopy. Mentink et al. (Dev Cell 2014) found that QR.pa cell migration stops upon expression of the Wnt receptor MIG-1, which surprisingly is not induced by positional clues but by a position-independent timing mechanism. We thus wondered 1) how robust the final QR.pax positioning was when confronted to stochastic noise and environmental variation and 2) how the final position and the underlying positioning mechanisms evolve. We measured variation in the final position of the QR.pax in a set of C. elegans wild isolates in different environments. Preliminary results indicate significant natural variation in QR.pax position in C. elegans, yet in a relatively tight window. At higher temperature, QR.pax mean position is posteriorly shifted, while starvation immediately after hatching increases the variance in QR.pax position. We further studied QR.pax position in different nematode species. In Caenorhabditis briggsae and C. tropicalis (the two other selfing species in the Caenorhabditis genus), the QR.pax cells are found in a similar position as in C. elegans. We aligned mig-1 putative cis-regulatory sequences in a large set of Caenorhabditis species (from http://caenorhabditis.org/, thanks to Mark Blaxter's laboratory). The alignment revealed highly conserved motifs in the upstream sequence and the first intron, especially in the Elegans supergroup, with some occasional losses in a species (e.g. C. briggsae). These motifs could provide crucial information about the evolution of mig-1 regulation and QR neuroblast migration.

P-192 Analysis of mechanisms underlying the metabotropic signal mediated by the Adhesion GPCR LAT-1 in oriented cell division

Lidia Duplice Simone Prömel, et al.

Rudolf Schönheimer Institute of Biochemistry, Medical Faculty, Leipzig University, Leipzig, Germany

Orientation of spindles and cell division planes is vital for tissue formation and cellular communication in many species. Especially during embryogenesis it ensures that correct cell-cell contacts are established, correct shapes are generated and an organism can develop. Although several signaling pathways in oriented cell division have been

well characterised such as wnt/frizzled, there is strong evidence for additional signal pathways especially in controlling early anterior-posterior polarity decisions. Recently, we have identified the homolog of the Adhesion G protein-coupled receptor Latrophilin, LAT-1, as a novel player in oriented cell division in the early C. elegans embryo. The cell surface receptor controls the proper anterior-posterior direction of cell division of specific blastomeres by a classical G protein-cascade based on coupling of the receptor to a Gs protein (GSA-1) which leads to elevated intracellular levels of the second messenger cyclic AMP (cAMP). Thus, strikingly, a metabotropic signal controls distinct aspects of polarity. We are combining in vitro and in vivo approaches to elucidate how LAT-1 mediates anterior-posterior cell division plane orientation via a non-polarised signal and by which mechanisms polarity information is elicited. We identified the downstream effector of cAMP by testing specific effector compounds for their ability to generate a phenocopy of the lat-1 mutant which displays defective embryonic cell division plane orientations in ABal descendants. Subsequent analyses using a specific cAMP biosensor shed light on a possible polarised cellular localisation of the effector and spatiotemporal dynamics of cAMP. Further, epistasis assay will identify further molecules involved in the LAT-1 signaling pathway. Taken together, our data contribute to address the question how a metabotropic signal can mediate a polarised process.

P-194 Unraveling the regulatory network of a complete C. elegans neural lineage

Euclides Fernandes Póvoa, Annabel Ebbing, Lorenzo Rella, Marco Betist, Hendrik Korswagen

Hubrecht Institute - Royal Netherlands Academy of Arts and Sciences (KNAW) and University Medical Center Utrecht

Development of metazoan nervous systems encompasses the generation, positioning and connection of diverse types of neurons in order to form highly organized neural circuitries. Our current knowledge shows that transcription factors (TFs) govern the molecular mechanisms that underlie different neural development phases such as progenitor cell proliferation, cell migration and lineage commitment. However, comprehensive in vivo characterization of such regulation in a complete neuronal lineage is still scarce. In the nematode Caenorhabditis elegans, the QR neuroblast lineage arises from a stem cell-like progenitor and undergoes a long anteroposterior migratory process to give rise to three final neuronal descendants: two sensory neurons (AQR and AVM) and one interneuron (SDQR). We are using this single cell model system to deepen our knowledge on how a complete neuronal lineage -including its migration and differentiation into different neuronal subtypes - can be regulated at the transcriptional level. We have combined FACS-based Q neuroblast sorting and RNA-sequencing (CEL-seq) to better understand the temporal transcriptional dynamics occurring in the QR lineage. Based on the data generated, we have identified hundreds of TFs that are expressed in the QR lineage. We selected a list of 26 candidates that were upregulated in the QR lineage compared to the progenitor cells, and which we are currently characterizing for their roles in neural lineage commitment and progression, neuroblast migration, and neuronal differentiation.

P-196 Characterizing mechanisms of neuronal plasticity at single-cell resolution in the dauer-exiting Caenorhabditis elegans larva

Friedrich Preußer, Ella Bahry, Stephan Preibisch

Max Delbrück Center For Molecular Medicine, Berlin Institute of Medical Systems Biology

Upon unfavourable conditions during its L1 stage, C. elegans can develop into the dauer diapause larva, an alternative developmental stage characterized by distinct morphology and behaviour. Once conditions improve, larvae actively reverse dauer-specific behavioural and morphological traits and resume reproductive development. Whereas genes involved in dauer formation have been previously identified, the neuronal dynamics during dauer exit as well as resulting transcriptional changes remain unknown at the single-cell level. Hence, our project aims at deciphering the precise neuronal activity patterns that drive the dauer exit decision, focussing on amphid neurons capable of sensing improved environmental conditions. To characterize the behavioural phenotype of dauer larvae in comparison to non-dauer larvae as well as to identify behavioural changes that succeed dauer exit, we developed a custom, easy-to-use imaging tool (the WormObserver) that allows tracking of larvae exiting the dauer stage over several hours. To further understand how such changes in behaviour are recapitulated by a change in neuronal activity patterns, we are employing lines expressing pan-neuronal GCaMP fusion proteins as well as three-

dimensional fluorescence microscopy. Hence, we are able to track the activity of individual neurons over the time course of dauer exit in response to dauer-exit inducing stimuli. To reproducibly characterize neuronal activity, we are developing custom microfluidic devices, which allow for precise delivery of these stimuli in a controlled environment. Furthermore, graph matching algorithms developed in our group will allow us to map all light-microscopy acquisitions of dauer larvae to a previously generated transmission electron microscopy dataset, which will significantly facilitate neuron identification and give important insights into the dauer larva connectome. Taken together, we are aiming at an in toto description of the dauer exit at the single cell level, focussing on how changing neuronal activity patterns are able to robustly drive complex developmental plasticity in a multicellular organism.

P-198 Investigating the role of Calcium signaling mediators in Caenorhabditis elegans lifespan regulation by signals from the germline

Laura Buck[1], James Moresco[2], John Yates[2], Cynthia Kenyon[3], Hildegard Mack[1]

[1]Institute for Biomedical Aging Research, Leopold-Franzens-Universität Innsbruck, Innsbruck, Austria

[2]Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA, USA

[3]Calico Life Sciences, South San Francisco, CA, USA

In Caenorhabditis elegans, reduced insulin/IGF-1 like signaling prolongs lifespan by activating the conserved transcription factor DAF-16 (FOXO). Loss of germ cells exerts similar life-prolonging effects and further doubles the already-prolonged lifespan of insulin/IGF-1 pathway mutants. While the mechanisms of DAF-16 regulation by signals from the insulin/IGF-1 like receptor DAF-2 are well characterized, the regulatory pathways activating DAF-16 in the absence of germline stem cells have not yet been fully elucidated. Using coimmunoprecipitation and mass-spectrometry, we obtained evidence for mediators of Calcium signaling differentially interacting with DAF-16 in wildtype and/or germline-deficient glp-1(-) worms. Consistent with published data, we observed that genetic mutations changing the activity of these Ca2+ signaling mediators extend the lifespan of wildtype worms. However, the extended lifespan of glp-1(-) animals is increased to a lesser extent. These data are consistent with the notion that longevity-promoting mechanisms depending on Ca2+ signaling mediators are already partially active in glp-1(-) worms. Currently, we are investigating the molecular mechanisms through which Ca2+ signaling mediators modulate C. elegans lifespan in greater detail.

P-200 Germline stem cells behaviour and differentiation in filarial nematodes, two processes under the endosymbiont bacteria Wolbachia control

Mercedes M. Pérez-Jiménez[1, 2, 3], Vincent Foray[1, 3], Nour Fattouh[1], Frederic Landmann[1]

[1]Centre de Recherche de Biologie cellulaire de Montpellier CRBM / CNRS, Montpellier, France

[2]Centro Andaluz de Biología del Desarrollo CABD / UPO, Sevilla, Spain

[3]These authors contributed equally to this work

Filariasis are diseases extremely debilitating in humans, and are lethal in cats and dogs, caused by filarial nematodes. From eradication of neglected tropical diseases to marketable veterinary drugs, interests converge toward Wolbachia bacteria. The mutualism of Wolbachia with parasitic filarial nematodes, like Brugia malayi, a causing agent of Elephantiasis, has triggered a resurgence of interest for this endosymbiont. While Wolbachia are present in somatic tissues, they only colonize the female germline to be passed onto the next generation. Wolbachia depletion induces female sterility and eventually kills the adult worms, unlike current anti-helminth drugs. However, how Wolbachia control the worm's fertility and survival is still a mystery. Massive apoptosis in embryos was reported as the first consequence of Wolbachia-depletion in B. malayi. We postulated a major role of Wolbachia during oogenesis because of their heavy colonization of the ovaries. Using novel approaches and techniques to deal with these large and unwieldy nematodes, we explored the making of an egg in filarial species, focusing on defects occurring during gamete production after Wolbachia depletion, to determine which key cell mechanisms are controlled by Wolbachia during normal development. Our data suggest that Wolbachia influence the female germline in a cell-autonomous manner as early as the germline stem cell pool maintenance, and stimulate the proliferation in parallel of the known key controllers. We will present the cellular defects following Wolbachia

depletion and suggesting that the endosymbionts are essential to maintain a proper germline developmental program in order to produce viable eggs and embryos.

P-202 Genetic mechanisms regulating TWK-28, a potassium channel controlling the locomotion of Caenorhabditis elegans

Noura Zariohi[1], Marie Gendrel[2], Alice Leclercq-Blondel[1], Alice Peysson[1], Thomas Boulin[1]

[1]Institut NeuroMyoGène - INMG CNRS UMR 5310 - INSERM U1217 - Université Claude Bernard Lyon 1 8 avenue Rockefeller 69008 LYON France

[2]École Normale Supérieure Paris (ENS) 45 Rue d'Ulm 75005 Paris France

Two-pore domain potassium channels (K2P) form a large family of conserved ion channels that play a central role in the establishment and maintenance of the resting membrane potential. They regulate neuronal excitability, hormone secretion, respiratory and cardiac functions. Despite the fundamental role of K2P channels, many questions about their biology are still unexplored. In particular, we still know little about the molecular and cellular processes that determine the number of active channels and their distribution at the cell surface. To address this simple yet fundamental question, our group uses the nematode C. elegans to discover genes and cellular pathways that control the biology of K2P channels in their native cellular context. The goal of my thesis project is to identify the molecular factors and cellular mechanisms that are required for the biosynthesis, trafficking and function of the TWK-28 channel. TWK-28 is expressed in body wall muscle and is required for the proper locomotion of C. elegans. A direct way to identify genes that are required for the function of the TWK-28 channel is to perform genetic screens for gene mutations that suppress the phenotype of hyperactive channel mutants. By combining protein structure data and two-electrode voltage-clamp electrophysiology in Xenopus oocytes, we have recently identified a key conserved amino acid that can be mutated to strongly increase the activity of vertebrate and invertebrate K2P channels. By inserting these mutations into the C. elegans twk-28 gene, we have been able to generate a new mutant strain with a striking behavioral phenotype. This “gain of function” mutation causes a very strong locomotor defect that was an ideal starting point for a genetic suppressor screen based on recovery of locomotion. We isolated 191 independent suppressor mutants. To rapidly clone the candidate mutants, we will take advantage of whole-genome sequencing technology. Candidate genes will then be validated by standard genetic tests.

P-204 Investigating RACK-1's role in regulating stem cell proliferation in the C. elegans germ line

Kara Vanden Broek[1], Chris Wang[1, 2], Xin Wang[1], Dave Hansen[1]

[1]University of Calgary, Calgary, AB, Canada

[2]Ambrose University, Calgary, AB, Canada

Stem cells possess the ability to either proliferate (form daughter cells that remain stem cells) or differentiate (form other cell types). Proper development requires a precise balance between stem cell proliferation and differentiation. The complete mechanisms by which this balance is established and maintained are not completely understood. We previously identified the protein kinase, CK2, as a negative regulator of stem cell proliferation in the C. elegans germline (Wang et al., 2014). We propose that CK2 functions in the GLD-1 pathway to inhibit proliferation and/or promote meiotic entry. Proteomic analysis identified RACK-1, the C. elegans ortholog of mammalian Receptor of C Activated Kinase, as a potential CK2 target protein. Initial analyses suggest that, like CK2, RACK-1 is a negative regulator of germline proliferation. RACK-1 has been shown to function as a scaffold protein in complexes such as the 40S ribosome (Link et al., 1999, Sengupta et al., 2004 and Nilsson et al., 2004) and the mRNA-induced silencing complex (Jannot et al., 2011). Therefore, RACK-1 may function to bridge the interaction between CK2 and its phosphorylation targets resulting in regulation of germline proliferation. Alternatively, RACK-1 may require direct phosphorylation from CK2 for its normal function in regulating proliferation. Investigating RACK-1’s role in the germline, and RACK-1’s interaction with CK2, will provide novel insight into how the balance between stem cell proliferation and differentiation is maintained in the C. elegans germline.

P-206 Insulin signaling and the age of the mothers modulate L1 arrest and recovery

María Olmedo[1], Alejandro Mata-Cabana[1], María Jesús Rodriguez-Palero[2], Antonio Fernández-Yáñez[2], Sabas García-Sánchez[1], Martha Merrow[3], Marta Artal-Sanz[2]

[1]Department of Genetics, University of Sevilla

[2]Andalusian Center for Developmental Biology, CSIC/JA/Pablo de Olavide

[3]Institute of Medical Psychology, Ludwig-Maximilians-Universität Munich

Living organisms constantly face changes in nutrient availability. In C. elegans, the progression through postembryonic development requires feeding, and animals arrest at the first larval stage (L1) if they hatch in the absent of food. This arrest involves quiescence of the blast cells that would normally divide to progress to L2. During L1 arrest, larvae undergo a process of ageing, as manifested by the accumulation of protein aggregates, increased ROS accumulation and mitochondrial fragmentation. The addition of food to arrested L1 leads to the reversion of these ageing markers and the resumption of larval development. Interestingly, the duration of L1 arrest affects the subsequent larval development and, after extended L1 starvation, animals take longer to reach adulthood. Using a quantitative method to measure each stage of development, we have found that delayed adulthood after extended arrest results mostly from an extended recovery time. Starvation time has only a minor effect in the rest of the developmental process, from L2 to adulthood. Upon feeding, L1 blast cell divisions proceed normally but they are delayed in animals that underwent extended starvation. We have observed that insulin signaling, which has a prominent role in the regulation of ageing, also modulates L1 ageing markers and recovery time after extended starvation. These findings suggest that insulin signal modulates the progression of L1 ageing. A recurrent observation for both recovery time and for the markers of ageing is that extended starvation results in increased variability. We have found that the age of the mothers introduces phenotypic variability in terms of resistance to L1 arrest. Larvae from older mothers recovered significantly faster and accumulated less ageing markers than those of younger mothers.

P-208 A new, general regulator of programmed cell death in C. elegans

Nadin Memar[1], Ryan Sherrard[1], Ralf Schnabel[2], Barbara Conradt[1, 3]

[1]Cell and Developmental Biology, Department Biology II, Ludwig-Maximilians-University Munich, Germany

[2]Technische Universität Braunschweig, Institute for Genetics, Braunschweig, Germany

[3]Center for Integrated Protein Science Munich – CIPSM, Department Biology II, Ludwig-Maximilians-University Munich, Germany

The genetic pathway required for programmed cell death during C. elegans development is highly conserved; however, its regulation is still not fully understood. In this study, we used temperature sensitive (ts) embryonic lethal mutants to identify genes that are not only required for programmed cell death but also for embryonic development. Using this approach, we identified the mutation t3443ts. Animals homozygous for t3443ts exhibit a partial block in programmed cell death during development. For example, 63% of the cells programmed to die during the first wave of embryonic cell death inappropriately survive. This block in cell death is not restricted to a specific cell lineage or cell death wave indicating that t3443ts defines a gene that acts as a general regulator of programmed cell death. To determine whether t3443ts affects the transcriptional upregulation of the BH3-only gene egl-1, the key activator of programmed cell death during development, we quantified the number of egl-1 transcripts in one specific cell death lineage, the MS lineage. We found that in 100% of t3443ts embryos analyzed, egl-1 transcripts are detectable at wild-type levels in the mother cell (MSpaap). In contrast, in the daughter cell programmed to die (MSpaapp), egl-1 transcripts are detectable at wild-type levels in only 27% of t3443ts embryos analyzed. Based on these findings we propose that the BH3-only gene egl-1 is misregulated in t3443ts embryos and that this misregulation leads to the inappropriate survival of cells programmed to die. Hence, t3443ts defines a new, general regulator of programmed cell death in C. elegans.

P-210 Peroxisomal Retrograde Signaling in Caenorhabditis elegans

Elisabeth Rackles, Stéphane G. Rolland

Cell and Developmental Biology, Department Biology II, Ludwig-Maximilians-University Munich, Germany
Peroxisomes are essential organelles required for several anabolic and catabolic pathways such as the beta-oxidation of very long chain fatty acids. Defects in peroxisomal biogenesis have been associated with severe diseases, called peroxisomal biogenesis disorders. The maintenance of functional peroxisomes is therefore essential. While there are some evidences that the peroxisomal Lon protease participates in quality control of peroxisomal matrix proteins, how peroxisomal quality control is regulated remains elusive. Quality control of other organelles has been shown to involve a retrograde signaling from the organelle to the nucleus to activate the production of organelle-specific proteases and chaperones in response to organelle-specific stress. For the first time, we present evidences of the existence of such a retrograde signaling for peroxisomes in Caenorhabditis elegans. We define this new signaling pathway as the peroxisomal retrograde signaling. Specifically, we show that perturbing peroxisomal biogenesis by inactivating genes encoding peroxisomal biogenesis factors leads to the transcriptional activation of the peroxisomal Lon protease (lonp-2). Furthermore, we show that perturbing peroxisomal biogenesis also activates the transcription of the peroxisomal catalase (ctl-2), which is required for eliminating toxic H2O2. Finally, we show that the transcriptional activation of lonp-2 and ctl-2 is dependent on the nuclear hormone receptor NHR-49, a functional homolog of the mammalian peroxisome proliferator-activated receptor alpha. We hypothesize that the peroxisomal retrograde signaling mediates the transcriptional activation of the peroxisomal Lon protease and catalase in response to peroxisomal stress, in order to maintain peroxisomal function.

P-212 Novel role for GSA-1, a heterotrimeric G protein alpha subunit, during ovulation in the C. elegans spermatheca

Perla Castaneda, Erin Cram
Northeastern University
The Caenorhabditis elegans (C. elegans) spermatheca, the site for embryo fertilization, is a tube composed of a single layer of cells that undergo cyclic stretching, constriction, and relaxation as ~150 oocytes pass through the gonad and into the spermatheca. The spermatheca consists of the distal neck, the bag, and the spermatheca-uterine (sp-ut) valve. Immediately after oocyte entry, the sp-ut valve constricts. After ~10 minutes, the bag begins to constrict, and the valve relaxes allowing the fertilized embryo to exit the spermatheca and enter the uterus. Upon oocyte entry, the phospholipase PLC is activated and inositol trisphosphate (IP3) and diacylglycerol (DAG) are produced. IP3 signals the release of calcium from the ER, which, coupled with Rho signaling and myosin activation, ultimately results in cell contraction in the spermathecal bag. Though a candidate RNAi screen, we have identified gsa-1, a heterotrimeric G-protein alpha subunit of the Gs class, as a novel regulator of spermathecal contractility during ovulation events. Depletion of gsa-1 through RNAi results in the inability of embryos to exit the spermatheca. This resembles what we have observed in worms with depleted plc-1. Using GCaMP3, a genetically encoded calcium sensor, we observed little to no calcium signal in the bag but strong calcium signal in the sp-ut valve. Expressing a GSA-1(GF) results in the initiation of calcium signaling in the spermatheca the absence of oocyte entry, something we have never observed in WT animals. We are currently exploring downstream effectors, including adenylyl cyclase, and PKA, as well as possible roles for the beta and gamma subunits.

P-214 An optogenetic arrhythmia model to study catecholaminergic polymorphic ventricular tachycardia mutations

Marcial Alexander Engel[1, 2], Elisabeth Fischer[1, 2, 3], Alexander Gottschalk[1, 2], Christina Schüler[1, 2]
[1]*Buchmann Institute for Molecular Life Sciences, Goethe University, Max von Laue Strasse 15, D-60438 Frankfurt, Germany*
[2]*Institute of Biophysical Chemistry, Goethe University, Max von Laue Strasse 15, D-60438 Frankfurt, Germany*
[3]*Present address: University of Edinburgh, Centre for Integrative Physiology, Hugh Robson Building, George Square, Edinburgh, EH8 9XE, UK*
Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a condition of abnormal heart rhythm (arrhythmia), induced by physical activity or stress. Mutations in the Ca^{2+} release channel ryanodine receptor 2 (RyR2) or the Ca^{2+}

binding protein calsequestrin 2 (CASQ2) are linked to CPVT. We introduced CPVT inducing mutations into the pharynx of *Caenorhabditis elegans*, which we previously established as an optogenetically paced heart model (Fischer et al., 2017; Schüler et al., 2015). By electrophysiology and video-microscopy, we characterized mutations in *csq-1* (CASQ2 homologue) and *unc-68* (RyR2 homologue). *csq-1* deletion impaired pharynx function and caused missed pumps during pacing. Deletion mutants of *unc-68* and in particular the point mutant UNC-68(R4743C), analogous to the established human CPVT mutant RyR2(R4497C), were unable to follow 3.7 Hz pacing. They show progressive defects during long stimulus trains. The pharynx either locked in pumping at half the pacing frequency or stopped pumping altogether, possibly due to UNC-68 leakiness and/or malfunctional Ca^{2+} homeostasis in sarcoplasmic reticulum. Importantly, we could reverse this 'worm arrhythmia' by the benzothiazepine S107, establishing the nematode pharynx for drug screening. Currently, we are inserting several CPVT inducing mutations into *unc-68* and are characterizing their effect on pharynx pumping. In addition to a specific drug screening this will allow us to investigate effects of known or new CPVT mutations.

P-216 Tissue-specific degradation of essential centrosome components reveals distinct microtubule populations at microtubule organizing centers

Maria Sallee, Jennifer Zonka, Taylor Skokan, Brian Rafetry, Jessica Feldman

Department of Biology, Stanford University, Stanford, CA, USA

The centrosome is the best characterized microtubule organizing center (MTOC), and is critical for microtubule organization in dividing animal cells. However, most differentiated cells establish MTOCs at non-centrosomal sites (ncMTOCs), yet how these ncMTOCs regulate microtubule organization and dynamics is largely unknown. The essential centrosomal microtubule nucleators γ-tubulin ring complex (γ-TuRC) and AIR-1/Aurora A both localize to the apical ncMTOC of intestinal epithelial cells in C. elegans embryos (Feldman and Priess 2012). Therefore, we tested the hypothesis that γ-TuRC and AIR-1 are also required for microtubule nucleation and organization at this apical ncMTOC. We first optimized the ZIF-1/ZF degradation system (Armenti et al. 2014) to allow intestine-specific depletion of AIR-1 and γ-TuRC. Using this method, we find that depleting the core γ-TuRC component GIP-1/GCP3 disrupts the apical localization of other γ-TuRC members including MZT-1/Mozart1, which we characterize for the first time in animal development. This result suggests that γ-TuRC localizes as a complex at the apical ncMTOC, as it does at the centrosome. We also observe that AIR-1 and MZT-1 are required to recruit γ-TuRC to the centrosome in dividing intestinal cells as expected. Surprisingly, AIR-1 and MZT-1 are not required to recruit γ-TuRC to centrioles or to the apical ncMTOC, indicating that different regulators control apical MTOC function and activity. Further, we find that the general organization of microtubules at the apical ncMTOC is not affected by co-depletion of γ-TuRC and AIR-1, nor by depletion of other microtubule regulators including TPXL-1/TPX2, PTRN-1/CAMSAP, or NOCA-1/Ninein. However, loss of GIP-1 and AIR-1 does remove a subset of dynamic EBP-2/EB1-marked microtubules. Together, these results suggest that different MTOCs use discrete proteins for their function, and that the apical ncMTOC is composed of distinct populations of microtubules that compete for a limited pool of resources.

P-218 A regulatory network of the GATA factor elt-1, Hox genes ceh-13, nob-1, and Wnt effectors pop-1 and sys-1 regulates early lineage patterning in C. elegans embryos

Amanda L. Zacharias[1, 2], Elicia Preston[2], Teddy D. Lavon[2], Barrington Alexander Bennett[2], Shaili D. Patel[2], Ilona Jileaeva[2], John Isaac Murray[2]

[1]*Division of Developmental Biology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*

[2]*Department of Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA*

The Wnt signaling pathway activates many target genes in the developing C. elegans embryo through its effectors POP-1/TCF and SYS-1/β-catenin. To understand how Wnt activates distinct target genes in different signaled cells, we investigated the regulation of two Wnt targets: ceh-13/Hox1 and nob-1/Hox13. Unlike in most animals, expression of these Hox genes overlaps, with ceh-13 expressed one cell cycle before nob-1 in several posterior daughter lineages at the 24-cell stage. We identified enhancers that control this early embryonic expression of ceh-

13 and nob-1, including several with overlapping expression, suggesting they are shadow enhancers. ceh-13 and the Hox co-factor unc-62/homothorax activate nob-1 expression in the ABp(l/r)ppp sublineages through two enhancers, but a third nob-1 enhancer active in this lineage does not require ceh-13 or unc-62, suggesting the use of multiple regulatory strategies in these overlapping enhancers. We identified elt-1 as a regulator of nob-1 expression in a distinct set of ABp sublineages (ABp(l/r)ap). elt-1 is known to specify terminal hypodermal fate, but its regulation of nob-1 occurs prior to the birth of pure hypodermal lineages. Each nob-1 enhancer has multiple predicted binding sites for ceh-13 or elt-1 and for pop-1. This suggests that ceh-13 or elt-1 provide context information to define which lineages express nob-1, while POP-1 and SYS-1 further limit nob-1 expression to Wnt-signaled posterior daughter cells. These results indicate that nob-1 is regulated by different combinations of context transcription factors in different sublineages. Our observation of an anterior Hox gene positively regulating expression of a posterior Hox gene to specify extreme posterior structures is novel. It appears that in C. elegans, Hox genes have acquired novel functions beyond specifying positional identity, suggesting animal body plans may be more flexible than previously appreciated.

P-220 Atlas of cell shapes, actomyosin activity and cell-cell adhesion in C. elegans early embryogenesis.

Francesca Caroti, Rob Jelier

KU Leuven

Understanding how cells self-organize into complex multicellular systems is a fundamental challenge in biology. Embryogenesis is the ideal example with undifferentiated founder cells moving to build all the tissue types of the mature organism. We use early C. elegans embryogenesis as a model, which is characterized by fast rounds of cell division and highly predictable, but adaptive, cell migration. To understand the contributions of cellular force generation, cell shape and cellular adhesions to the positioning of cells we visualized F-actin, non-muscular myosin, cell membranes and E-cadherin with fluorescent proteins and studied their dynamics by in vivo imaging. We are processing the lineages to make an atlas of cell shapes, their cellular adhesion and force generation. Early analysis highlights that actomyosin activity and fast dynamic cell deformation point to active movements in only specific cells. In general, F-actin is not equally present among cells and it seems to be differentially localized based on the cell fate. E-cadherin (HMR-1) distribution is ubiquitous early in embryogenesis, except for a cluster of cells whose cell shapes still suggest strong adhesion, which points to the presence of alternative cellular adhesion molecules. In later stages (6th AB generation), differential adhesion can be observed, with cell-cell adhesion foci between cells moving together. The atlas will serve as a reference for further studies to identify new proteins regulating cell-cell contact and cell migration as well as for biophysical models for cellular positioning.

P-222 A short helix in the C-terminal region of dynein light intermediate chain links the motor to structurally diverse adaptors for cargo transport

Cátia Carvalho[1, 2], Ricardo Celestino[1, 2], José Bernardo Gama[1, 2], Reto Gassmann[1, 2]

[1]*i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal*

[2]*IBMC - Instituto de Biologia Molecular e Celular, Porto, Portugal*

All animal cells use the molecular motor cytoplasmic dynein 1 (dynein) to transport proteins, mRNA, and organelles towards microtubule minus ends. How dynein is recruited and locally activated on cargo remains poorly understood. Many cargo-specific adaptors for dynein have been shown to bind to the disordered C-terminal region of dynein light intermediate chain (LIC), an essential subunit of the motor. An important unresolved question is how adaptors bind to the same LIC region and the functional role of this interaction in cargo transport. Using binding assays with purified proteins, we show that a conserved C-terminal helix in LIC (helix 1) is essential for the interaction with diverse cargo adaptors. To study the function of helix 1 in vivo, we used CRISPR/Cas9-mediated genome editing to introduce mutations into the C. elegans LIC homolog dli-1 that disrupt binding of DLI-1 to adaptors. Point mutations in the DLI-1 helix 1 cause sterility, shorten lifespan, and impair locomotion, consistent with previous phenotypes associated with dynein mutants. We also directly examined the distribution and transport kinetics of early endosomes in axons and found them to be severely perturbed in helix 1 mutants, identical to what is observed when

the entire DLI-1 C-terminal region is deleted. We are also analyzing the distribution of mitochondria, autophagosomes and synaptic vesicles in axons to determine whether helix 1 is required for the transport of different types of cargo. Additionally, we have deleted a second helical segment at the DLI-1 C-terminus (helix 2), which is also conserved, but, based on in vitro assays, is less important for adaptor binding. We are currently characterizing how deleting helix 2 affects dynein-dependent processes in mutant animals. In summary, we find that a conserved helix in the intrinsically disordered region of LIC links the motor to diverse cargo and is essential for dynein function in vivo.

P-224 C. elegans as a model to investigate mitochondrial disorders

Valeria Morbidoni, Cristina Cerqua, Maria Andrea Desbats, Leonardo Salviati, Eva Trevisson

Dipartimento di Salute della Donna e del Bambino, Università degli Studi di Padova e Istituto di Ricerca Pediatrica, IRP, Città della Speranza, Padova.

A growing number of diseases are associated with an altered function of the mitochondrial oxidative phosphorylation (OXPHOS). Around 60-80% of human genes have a corresponding orthologue in C.elegans (1) and many cellular pathways are conserved between nematodes and higher organisms, making it a useful in vivo model. A valuable tool in the analysis of gene function in worms is genetic interference mediated by double-stranded RNA (RNAi) (2). Using this approach we silenced a series of genes required for cytochrome c oxidase (COX) assembly and performed an extensive phenotypic characterization of silenced animals. A COX-specific histochemical staining in worms allowed us to demonstrate that these genes are essential for COX activity (3), thus establishing multicellular models of COX deficiency. We aimed at analyzing the presence of OXPHOS complexes in silenced animals through blue native polyacrylamide gel electrophoresis (BN-PAGE). A constraining factor is the efficient isolation of mitochondrial proteins which is complicated by the extremely resilient worm exoskeleton. We therefore optimized a protocol previously developed (4) to analyze mitochondrial respiratory complexes in COX-deficient worms. Our findings showed that C.elegans can be efficiently used to model mitochondrial disorders. We are now planning to employ these animals to test novel therapies. (1) Kaletta T, Hengartner MO. Nat Rev Drug Discov. 2006 May;5(5):387-98. (2) Timmons L, Court DL, Fire A. Gene. 2001 Jan 24;263(1-2):103-12. (3) Cerqua C, Morbidoni V, Desbats MA, Doimo M, Frasson C, Sacconi S, Baldoin MC, Sartori G, Basso G, Salviati L, Trevisson E. Biochim Biophys Acta. 2018 Jan 30;1859(4):244-252 (4) van den Ecker D, van den Brand MA, Bossinger O, Mayatepek E, Nijtmans LG, Distelmaier F. Anal Biochem. 2010 Dec 15;407(2):287-9.

P-226 Inferring gene regulatory network governing early ageing process in C. elegans

Manusnan Suriyalaksh[1], Marta Sales Pardo[2], Nicolas Le Novere[1], Olivia Casanueva[1]

[1]*The Babraham Institute*

[2]*Department of Chemical Engineering, Universitat Rovira i Virgili*

C. elegans is a perfect model organism for aging studies due to its short lifespan and expansive genetic information available. Studies on the model have uncovered key ageing pathways, along with revealing to us the staggering complexity of the process. The complexity, thus, invites a top-down systems approach to pinpoint central systems affecting ageing. Our goal is to characterize the complex systems of gene expression during early adulthood in C. elegans utilizing network inference. With timely-resolved (at four hour intervals) transcriptomics data of long-lived and normal-lived C.elegans, along with the wealth of –omics data publicly available, we integrate the data to construct an evidence-based gene regulatory network. Next is to identify promising algorithms that infer causal relationships to best depict the crucial moment triggering ageing process. Recent developments have shown that utilizing wisdom of the crowd and incorporating extensive priors information vastly increases the accuracy of the predictions. Our efforts aim at resolving the first changes that begin the ageing process in worms and to later also understand if similar molecular mechanisms influence ageing in humans.

P-228 Uncovering the role of the scaffold apical PAR proteins in established epithelial tissues

Helena Pires, Victoria G. Castiglioni, Jana Kerver-Stumpfova, Mike Boxem
Division of Developmental Biology, Department of Biology, Faculty of Science, Utrecht University, The Netherlands
The apical and basolateral domains of epithelial cells are distinguished by a different assembly of proteins and lipids, allowing distinct roles in spatially different regions of the cells. To create these functionally different compartments, epithelial cells rely on an organized distribution of cortical polarity proteins. These proteins have an essential role in positioning essential components of different cell pathways in distinct cellular subdomains. The molecular structure of cortical polarity proteins indicates a scaffolding function. Indeed, polarity proteins have an extensive network of different binding partners through which they integrate cues from the surrounding microenvironment and control a multitude of cellular processes. In our work, we aim to understand the biological role of the PAR apical complex in established epithelial tissues. Our knowledge on how the PAR apical polarity proteins intersect with other epithelial cell pathways, and how this intersection modulates the different pathways and regulates the polarity network itself, is still very incomplete. A major problem comes from the fact that the cross-talk between the polarity proteins and a variety of different cell pathways, vary from one context to another and from tissue to tissue. In addition, the PAR proteins are essential during embryonic development, which complicates the task of dissecting the role of these proteins in established tissues. To circumvent those problems, we combined CRISPR and reversible protein degradation (Auxin Inducible Degradation), in order to uncover the role of the PAR apical complex in vivo and in specific established epithelial tissues at specific times of larval development. Here, we show the endogenous expression of PAR3 and PAR6 and how epithelial tissues function is affected in the absence of these proteins at different points of larval development.

P-230 M05D6.2, an ortholog of human T-complex protein 11 (TCP11), is necessary for sperm function and fertility in Caenorhabditis elegans

Emily Lopes, Amber Jacob, Danielle Cooley, Matthew Marcello
Pace University
Human t−complex protein 11 (TCP11) is a testis−specific gene product that is hypothesized to be necessary for proper sperm capacitation, acrosome reaction, and sperm morphology. TCP11 function is of clinical interest because human patients have identified with mutations in the gene encoding TCP11. Our goal is to investigate the function of the C. elegans ortholog of TCP11, M05D6.2, to understand the role of TCP11 in human reproduction. C. elegans sperm activation includes processes similar to sperm capacitation and acrosome reaction in mammals, and we hypothesize that M05D6.2 is necessary for proper sperm activation in C. elegans. We have used RNA interference (RNAi) to disrupt the gene function of M05D6.2 in C. elegans. Hermaphrodites subject to M05D6.2 RNAi−treatment show no reduction in fertility. However, when male C. elegans are subject to M05D6.2 RNAi−treatment our results indicate that they have a significant decrease in fertility, despite making a normal number of sperm. We have generated three transgenic C. elegans strains using CRISPR/Cas9 genome editing (a deletion mutant, a mutant mimicking mutations found in infertile male patients, and a GFP−tagged version of the protein) to further characterize M05D6.2 function and localization. We are also investigating C. elegans strains with single nucleotide polymorphisms (SNPs) in the gene to characterize the function of specific residues in the TCP11 domain.

P-232 Cellular response to Wnt signals: an in vitro approach using C. elegans embryonic cells

Pritha Pai, Pierre Recouvreux, Pierre-Francois Lenne
Institut de Biologie du Développement de Marseille, UMR 7288
Embryonic development is controlled by secreted proteins that signal at distance through specific receptors present on the plasma membrane of target cells. One such well-studied protein is Wnt that signals through its receptor Frizzled. In C. elegans, Wnt signalling controls various asymmetric divisions along the antero-posterior axis. However, the mechanisms responsible for the polarization of such target cells is still unknown. To understand these mechanisms, we use the primary culture of C. elegans embryonic cells and expose them to artificial Wnt gradients controlled by microfluidic devices. Using high-resolution imaging to visualize fluorescently tagged endogenous

Frizzled on the membrane, we monitor the localization and dynamics of the receptor. This approach allows us to determine how Wnt ligands induce polarization at the cellular level, which in turn can lead to asymmetric division.

P-234 An endomembrane-resident zinc transporter negatively regulates systemic RNAi in C. elegans.

Katsufumi Dejima, Rieko Imae, Yuji Suehiro, Shohei Mitani

Tokyo Women's Medical University

In C. elegans, dsRNA spreads over the whole body and leads to RNA silencing in a cell non-autonomous manner. This phenomenon is called systemic RNAi and can be used as a model for systemic functional RNA spreading. To date, several genes acting in systemic RNAi have been genetically identified. However, cellular pathways and molecules that mediate RNA transport between cells remain largely unknown. We previously showed that RSD-3/EpsinR is involved in import of silencing RNA, and the rsd-3 mutants display incomplete defects in systemic RNAi. Here we show analysis of suppressors for RNAi defects in the rsd-3 mutant and found that a zinc transporter functions as a negative regulator for dsRNA import. The mutant itself showed enhanced RNAi (Eri) phenotype but were fertile at 25 °C, excluding the possibility that it functions in the core endogenous RNAi pathway. Mutants for all other zinc transporter genes exhibited normal response to feeding RNAi, suggesting unique role of this transporter on systemic RNAi. This zinc transporter is widely expressed during development, acts in a cell non-autonomous manner, and is predominantly localized to late endosomes. Its null mutants showed smaller RAB-11-associated vesicles in the intestine, suggesting its involvement in membrane trafficking regulation. Epistasis analysis indicated that sid-1 and sid-2 but not sid-3 or sid-5 are essential for the Eri phenotype seen in this mutants. The expression of this transporter was enhanced in sid-5 but not in rsd-3 mutants. On the other hand, the zinc transporter mutants displayed altered SID-1 but not SID-2 distribution. Our data uncovered the zinc transporter as a novel cellular factor functioning in negative regulation of systemic RNAi.

P-236 Adhesion GPCRs in regulation of food intake and metabolism of C. elegans

Johanna Schön, Johanna Weinert, Daniel Matúš, Torsten Schöneberg, Simone Prömel

Rudolf-Schönheimer-Institute of Biochemistry, Medical Faculty, Leipzig University, Leipzig, Germany

Adhesion G protein-coupled receptors (aGPCRs) are the second largest class of GPCRs and involved in various essential biological processes, rendering them promising drug targets. However, their functions and especially their signalling mechanisms are poorly understood. In mammals increasing evidence highlights the involvement of several of these cell surface receptors in regulation of metabolic processes and maintaining energy homeostasis. However, their underlying mechanisms are insufficiently understood precluding an indepth evaluation of their impact on metabolic regulation. Compared to the 33 members in mammals, the genome of the nematode C. elegans harbours three aGPCRs, the Latrophilins lat-1 and lat-2 and the Flamingo/CELSR homologue fmi-1 facilitating analyses on the role of these receptors in metabolic processes. This study aims at characterising the role of aGPCRs in metabolism of C. elegans. Our data show that a lat-1 as well as a fmi-1 null mutants have a prolonged lifespan compared to wild-type individuals. For lat-1 we were able to show that this longevity is most likely due to caloric restriction. lat-1-deficient nematodes display a decreased pharyngeal pumping rate as well as reduced bacteria ingestion, suggesting that LAT-1 plays a role in food intake. In contrast, our analyses showed that the cause for increased lifespan in fmi-1 mutants is not caloric restriction but alterations in metabolic processes indicated by elevated fat levels and resistance to thermal stress. These data indicate that aGPCRs fulfil different functions in the regulation of food intake and metabolic regulation.

P-238 Examining promoter states at the single molecule level during transcription initiation

Jennifer Semple[1], Arnaud Krebs[2], Peter Meister[1]

[1]*Institut für Zellbiologie (IZB), University of Bern, Switzerland*

[2]*EMBL, Heidelberg, Germany*

Transcription initiation is a multistep process requiring changes in chromatin state, removal of nucleosomes and step-wise recruitment of transcription factors, the preinitiation complex and RNA polymerase to the gene promoter. Most genome-wide techniques to study the transcriptional state of promoters, such as DNase hypersensitivity, MNase digestion or chromatin IP, provide average population measurements. However, it is now well established that transcription is a highly dynamic process, and observed "average occupancy rates" can be produced by very different underlying distributions of discrete states. In order to investigate this, we use a single molecule fingerprinting approach, first developed in fly cells, to map protein-bound regions within individual promoters. In this method, applicable to organisms that do not have endogenous cytosine methylation, isolated cell nuclei are treated in vitro with bacterial CpG and GpC methylases. After purification, DNA is treated with bisulfite, converting unmethylated C to U. As a consequence, CpG and GpC sites initially bound by proteins are protected from methylation, converted to UpG and GpU, and can be identified using high-throughput sequencing. As a model system we are using dosage compensation of the X chromosome. In hermaphrodites, a condensin-like complex, the dosage compensation complex (DCC) is recruited to the X chromosome and down-regulates the expression of X- linked genes on both chromosomes in order to equal expression from the single X chromosome in males. Previous work has shown that the DCC regulates transcription at the level of transcription initiation. We are applying the dual-enzyme single molecule footprinting (dSMF) technique both genome-wide and to 500 bp amplicons covering the transcription start site of 48 genes on the X chromosome and 48 expression-matched genes on the autosomes.

P-240 Epigenetic mechanisms of nematode mouth-form plasticity

Michael Werner, et al.

Max-Planck Institute for Developmental Biology

Many animals and plants can respond to their environment by developmental plasticity, the ability to produce different phenotypes form the same genotype. In nematodes crowding and starvation regulate entry into an arrested dauer stage, and in some species an alternative mouth-form decision (bacterivorous vs. predatory). While forward genetic screens have elucidated many of responsible genes, the epigenetic mechanisms connecting the environment to these gene-switches is still lacking. We used the mouth form plasticity of Pristionchus pacificus as model to understand the molecular mechanisms of environmental influence. First, we established a set of culture conditions to easily tune bacterivorous vs. predatory mouth forms, and then performed RNA-seq at every major developmental stage in conditions that induce either mouth form. We have identified the identity and timing of large gene networks corresponding to each mouth form. We also performed temporal morphometric analysis to determine when the phenotypic decision is made. By combining these data with reciprocal transplant experiments, we identified a ~36 hr critical window between juvenile stage 2 and 4 in which the mouth form phenotype is susceptible to the environment. Finally, we profiled dynamic chromatin changes by ChIP-seq and ATAC-seq before, during, and after this critical window in bacterivorous and predatory-inducing conditions. Collectively, our results reveal changes in the chromatin landscape associated with environmental switches, and insight into the molecular mechanisms of phenotypic plasticity.

P-242 Investigating glia-to-neuron cell fate switches in C. elegans

Michele Sammut, Rachel Bonnington, Milou van der Lans, Kishan Khambhaita, David Elliott, Arantza Barrios, Richard Poole

Department of Cell and Developmental Biology, University College London

We have discovered two distinct cases in which sex-shared, differentiated glial cells undergo glia-to-neuron cell fate switches during sexual maturation in male but not hermaphrodite C. elegans. The first requires the asymmetric division of the AMso to produce the interneuron MCM and the second occurs through a direct cell fate switch of the PHso1 to sensory neuron PHD. To uncover molecular regulators of glia-to-neuron cell fate plasticity we performed a GFP based forward genetic screen and isolated nom ('No MCM') mutants in which MCMs fail to be specified. Using a battery of glial and neuronal markers we have identified mutants that affect sequential stages of MCM development Class I: AMso specification; Class II: AMso division; Class III: MCM neuronal differentiation and Class

IV: MCM neuronal subtype specification. We show that two AMso division mutants, nom-5 and nom-8 exclusively affect this glial cell division and are alleles of cdk-4, a key cell-cycle regulator that governs the G1-S decision. This suggests that specific regulation at the level of the G1-S transition may be key to this type of cell-fate switch. To explore the subsequent developmental events we use a temperature-sensitive lin-5/NuMA mutant and a battery of other cell cycle mutants to assess the role of DNA replication as well as the orientation of AMso cell division on neuronal fate. To explore the mechanisms of MCM specification after the AMso division we are now characterising Class III mutants. We hope that discoveries gained from these naturally occurring cases of glia-to-neuron cell fate switches, will provide insight into how particular cells retain plasticity during development, while others become increasingly restricted in their potential fate, a stubborn but central question in biology.

P-244 Evolutionary Stable Strategies of Signalling and Signal Interference in Competitive Ephemeral Environments

Arthur Hills[1], Mark Viney[2], Simon Harvey[1]

[1]Biomolecular Research Group, Department of Geographical and Life Sciences, Canterbury Christ Church University, Canterbury, UK

[2]School of Biological Sciences, University of Bristol, Bristol, UK

Public signals are present in a number of biological systems and used for a diverse range of functions including sexual attraction and repulsion, feeding behaviour, and developmental growth. Within Caenorhabditis elegans, public ascaroside signalling is used to regulate the formation of Dauer larvae, a developmental stage which facilitates migration between the ephemeral food patches in which it proliferates. To understand how such public signals may be used within environments where multiple individuals or genotypes compete over the same resources, we are studying a simple game theory model. The system consist of two identical players representing two genotypes, who decide whether to invest or avoid the environment on the sole basis of a chemical marker that can be uniquely detected by the individual players. Each player is able to interfere with the competitor for a cost by emitting the chemical marker their competitor detects. This is motivated by a competition cost to both players investing in the same environment. We examine the evolutionary stable strategies of this model to identify the range of conditions under which competitors may choose to or not to interfere. Additionally we identify systems where interference is used to inform a competitor not to compete.

P-246 Rewiring of the germ line transcriptional network in C. elegans through TE co-option

Francesco Nicola Carelli, Chiara Cerrato, Jürgen Jänes, Julie Ahringer

The Gurdon Institute and Department of Genetics, University of Cambridge, Cambridge CB2 1QN, United Kingdom

Transposable elements (TEs) are selfish DNA elements that can replicate and move to new locations in the genome. Their mobilization relies on the expression of their genes through host recognition of TE transcriptional regulatory elements. The significant overlap observed between TEs and putative regulatory elements in several eukaryotes suggests that expansions of repeat families might have provided the raw material to rewire transcriptional networks in different lineages, sometimes facilitating the evolution of novel structures. Despite the numerous genomic associations observed between TEs and regulatory sequences, the functional role of TEs remains largely untested, mostly due to technical limitations of the model systems investigated. In the present work, we investigate the association of TEs with germ line promoters in C. elegans. By analysing regulatory elements active at different developmental stages in wild type and mutant C. elegans strains, we identified sets of germ line-specific elements and their associated genes. We observed that this set of germ line-specific promoters was strongly and specifically associated with different classes of DNA transposons. Furthermore, sequence analysis highlighted the enrichment of two previously undescribed, closely-spaced motifs in germ line-specific promoters. Whereas ~50% of the germ line promoter-associated motif pairs were located in nonrepetitive DNA and showed strong signatures of evolutionary conservation, the remaining motifs were located within three main TE classes. Evolutionary comparisons indicate that at least one TE class specifically arose in the C. elegans lineage, suggesting that its amplification might have seeded the C. elegans genome with new germ line-specific regulatory regions. Further work

is currently under way to confirm the functionality of the motifs identified in this study and evaluate the role of the motif-associated TEs amplification in the regulatory evolution of gem line transcription in C. elegans and related nematode species.

P-248 Model-free analysis of spindle elongation trajectories in C.elegans one-cell embryo.

Yann Le Cunff[1], Laurent Chesneau[2], Sophie Theis[1], Valentin Costes[1], Sylvain Pastezeur[1], Jacques Pécréaux[2]

[1]IGDR, Université Rennes 1

[2]IGDR, CNRS

Cell division is a complex multi-scale process, in which molecular actors constantly interact. How a robust and faithful emerge from all these actors interacting over time is yet to be understood. To address this question, analyzing cell-to-cell variability is of utmost importance : do cells quantitatively differ from each other but only by a different dosage of the same underlying mechanisms or do they qualitatively differ from each other, i.e. other mechanisms come into the play ? While obvious in some examples, e.g. cell differentiation, it is generally challenging to identify whether a phenomenon observed is novel or only a variation of the wild type phenotype. We tackled this issue by studying spindle elongation during the division of the C.elegans one-cell embryo. Through a model-free analysis, we showed that, in a population of wild-type cells, three main independent mechanisms recapitulate more than 95% of cell-to-cell variability. The first mechanism mainly acts after anaphase onset and coincides with the spindle elongation, while the second starts in late metaphase and contribute to slowly elongating the spindle. Surprisingly, the third mechanism allows the spindle to retract prior to anaphase onset. We then investigated whether the same three mechanisms drive cell-to-cell heterogeneity over a wide range of genetic perturbations. All these perturbations – over 1500 experiments covering 100 genetic conditions - focused on impairing the most documented actors implicated in generating forces (microtubules, centrosomes, cortical anchors, kinetochores, spindle molecular motors,…). The same model-free analysis showed that cell-to-cell variability stems from the same mechanisms that we unveiled in the wild type cells. In a word, genetic perturbations only lead to quantitative differences (dosages) but not qualitative differences (no new mechanism appeared). To offer tentative predictions of the mechanisms involved in each perturbation, we mapped the cell-to-cell heterogeneity with different parametrization of mathematical models using agent-based simulations.

P-250 H3K4 methylation and chromatin organization in the C. elegans germline.

Marion Herbette[1], David Llères[2], Aymeric Bailly[3], Robert Feil[2], Valérie Robert[1], Francesca Palladino[1]

[1]Laboratoire de Biologie et Modélisation de la Cellule UMR5239, Lyon, France

[2]Institut de génétique moléculaire de Montpellier (IGMM) – CNRS : UMR5535, Montpellier, France

[3]Cell Biology Research Center of Montpellier, CNRS, UMR-5237, Montpellier, France

The structural and functional organization of mitotic and meiotic chromosomes is largely dependent on condensins, large multisubunit protein complexes conserved across species. However, depletion studies in several species including C. elegans, suggest that condensin is not essential for mitotic chromosome formation in vivo, although condensin-depleted chromosomes are decondensed and form massive chromatin bridges in anaphase. How additional players, including histones, contribute to chromatin condensation remains an open question that I am addressing. Our lab has previously shown that methylation of lysine 4 of histone H3 (H3K4me), a mark commonly associated with promoter regions of actively transcribed or poised promoters, plays a role in the establishment and maintenance of the C. elegans germline through the regulation of the germline transcriptional program and genome stability [1,2]. I have obtained evidence suggesting that, in addition to playing a role at promoters, H3K4 methylation may also contribute to global germline chromatin organization. Genetic and microscopy analysis shows that set-2/SET1, the major H3K4 methyltransferase in C. elegans, acts in parallel with condensin II to promote chromatin compaction. Experiments where the nanoscale chromatin compaction was assayed in set-2 mutants germlines by a quantitative FLIM-FRET approach [3] supports a role for H3K4 methylation in the compaction of germline nuclei. I will discuss currently ongoing experiments to further explore how H3K4 methylation and condensin II collaborate to

organize and compact chromatin in the C. elegans germline. [1] Herbette et al., 2017, DNA Repair (Amst), 57:139-150. [2] Robert et al., 2014, Cell Reports 9, 443-450. [3] Llères, Bailly et al., 2017, Cell Reports 18, 1791-1803

P-252 Nicotinamide-N-methyltransferase controls behavior, neurodegeneration and lifespan by regulating neuronal autophagy

Kathrin Schmeisser[1], Alex Parker[1, 2]

[1]*CRCHUM*

[2]*Department of Neuroscience, Universite de Montreal*

Nicotinamide N-methyl-transferase (NNMT) is an essential contributor to various metabolic and epigenetic processes, including the regulating of aging, cellular stress response, and body weight gain. Epidemiological studies show that NNMT is a risk factor for psychiatric diseases like schizophrenia and neurodegeneration, especially Parkinson's disease (PD), but its neuronal mechanisms of action remain obscure. Here, we describe the role of neuronal NNMT using C. elegans. We discovered that ANMT-1, the nematode NNMT ortholog, competes with the methyltransferase LCMT-1 for methyl groups from S—adenosyl methionine. Thereby, it regulates the catalytic capacities of LCMT-1, targeting NPRL-2, a regulator of autophagy. Autophagy is a core cellular, catabolic process for degrading cytoplasmic material, but very little is known about the regulation of autophagy during aging. We report an important role for NNMT in regulation of autophagy during aging, where high neuronal ANMT-1 activity induces autophagy via NPRL-2, which maintains neuronal function in old wild type animals and various disease models, also affecting longevity. In younger animals, however, ANMT-1 activity disturbs neuronal homeostasis and dopamine signaling, causing abnormal behavior. In summary, we provide fundamental insights into neuronal NNMT/ANMT-1 as pivotal regulator of behavior, neurodegeneration, and lifespan by controlling neuronal autophagy, potentially influencing PD and schizophrenia risk in humans.

P-254 Phospholipid translocase function regulates vesicle transport in specific ciliated chemosensory neurons.

Simon Tuck, Lars Nilsson, Shapour Rahmani, Ani Minasian, Manon Baures

UCMM, Umeå University, Sweden.

The two leaflets that make up membranes in eukaryotic cells are very often highly asymmetric with respect to the phospholipids they contain. This asymmetry is maintained in part by the action of P4-family ATPases that function as transamphipathic phospholipid translocases to catalyse the inward translocation of specific phospholipids from the exoplasmic to the cytoplasmic leaflet. C. elegans contains six tat genes, tat-1 – tat-6. tat-1 and tat-5 have been studied for their roles in regulating phagocytosis and embryonic development respectively but the functions of the remaining tat genes have not been described in detail previously. We have found that several of them have roles in vesicle transport in specific cells. In particular, we have found that tat-6 is expressed in a specific subset of gustatory neurons and that a TAT-6::GFP fusion protein accumulates in cilia. We have isolated both gain-of-function and loss-of-function mutants of tat-6 and found that, while tat-6 is not required for ciliogenesis, it has specific functions in vesicle transport within cilia. tat-6 mutants have behavioural defects consistent with the expression pattern suggesting that the transport defects affect cilium function. Outside of the nervous system, tat-6 expression is restricted to two cells in the somatic gonad, sujc, which form the central part of the spermatheca-uterine valve. Deregulation of tat-6 function in these cells, inhibits movement of germ cells through the valve.

P-256 The physiological role of acd-5 in dauer larvae formation and chemosensation in C. elegans

Laura Grundy, Eva Kaulich, William R Schafer

Division of Neurobiology, MRC Laboratory of Molecular Biology, Cambridge Biomedical Campus, Francis Crick Avenue, Cambridge CB2 0QH, UK

Acid-sensing ion channel proteins are members of the DEG/ENaC super-family, a large group of sodium channel subunits conserved across species. Some DEG/ENaC channels, for example MEC-4, are involved in mechanosensation in C. elegans; however, the in vivo functions of most of these channels remain poorly understood. Here we investigate the physiological role of acd-5 which was initially identified from a large screen of C. elegans micro-behaviours using automated recording and analysis, where the acd-5 mutants showed a subtle locomotion phenotype. Surprisingly, under normal food conditions, acd-5 expression is only observed throughout the intestine. Interestingly, under unfavourable growth conditions in the dauer larva stage, acd-5 is also expressed in the two ASI neurons which is known to inhibit entry into dauer stage. Given the temporal and spatial expression of acd-5 in dauer larvae, we hypothesised that acd-5 expression might regulate chemosensation in relation to dauer larvae formation. Indeed, our preliminary data suggest that in the presence of pheromones dauer arrest in acd-5 mutants is reduced in comparison to N2, and acd-5 mutants also tend to have a shorter lifespan. Furthermore, our preliminary experiments show chemosensory deficits in acd-5 mutants to lysine. Using calcium imaging, behavioural and genetic tools, we are currently investigating these phenotypes, ultimately aiming to investigate the physiological context and mechanism underlying acd-5 activation in dauer larvae formation and chemosensation.

P-258 Analysis of heterochromatin in repetitive element repression and ageing

Tessa Gaarenstroom[1], Alicia McMurchy[1], Przemyslaw Stempor[1], Alex Appert[1], Luke Harvey[1], Michael Schoof[1], Ni Huang[1], Andrea Frapporti[1], Matt Churgin[2], Christopher Fang-Yen[2], Julie Ahringer[1]

[1]Gurdon Institute, University of Cambridge, United Kingdom

[2]Department of Bioengineering, University of Pennsylvania, Philadelphia, United States

Heterochromatin is important for genome regulation and is required for transcriptional repression of genes and repeat elements. Heterochromatin in C. elegans is enriched on the repeat-rich chromosome arms, regions which are associated with the nuclear lamina. We previously found that several heterochromatic proteins, as well as H3K9 methylation, are important for repression of repetitive elements and genes. HPL-2/HP1, LIN-13, LIN-61, LET-418/Mi-2 and H2K9me2 histone methyltransferase MET-2/SETDB1 show genome-wide co-localization and enrichment at repetitive elements, and mutants derepress a subset of DNA transposons. Furthermore, these proteins also cooperate with small RNA pathways in repetitive element repression, thereby protecting against genotoxic stress (McMurchy et al, 2017). Since HPL-2 and LIN-61 contain domains that can bind H3K9me2/3, we utilized ChIP analyses to investigate the requirement for H3K9 methylation in the localization of heterochromatin proteins. Consistent with previous findings (Garrigues et al, 2015), we found that HPL-2 has a normal binding pattern in met-2 set-25 mutants, which lack all H3K9 methylation (Towbin et al, 2012). Similarly, we found that LIN-13 and LIN-61 also still localize to their target sites, indicating that H3K9 methylation is not necessary for chromatin binding. We are further studying how these heterochromatin proteins are targeted to specific sites in the genome, and whether there are different mechanisms for binding to genes or repeat subclasses. Loss of heterochromatin and derepression of transposable elements has been shown to correlate with ageing in multiple organisms. We found that heterochromatin mutants are shorter-lived than wild type. Coinciding with an acceleration in ageing, we observed that these mutants show premature disintegration of the nuclear lamina. Furthermore, transcriptional profiling revealed a progressive upregulation of repeats in ageing animals. By investigating the relative levels and genomic localization of heterochromatin modifications during ageing, and analyzing repeat expression, we aim to define how heterochromatin protects adult somatic cells from ageing.

P-260 Temporary Atresia and Corsetry are Essential for Worms to Molt

Hannah Maul-Newby[1, 2], Chloe Maybrun[2], Alison Frand[2]

[1]Department of Molecular, Cell and Developmental Biology, University of California, Santa Cruz

[2]Department of Biological Chemistry, School of Medicine, University of California, Los Angeles

Temporary appendages protect emergent epithelial organs and body systems from physical damage during key developmental transitions. While the medical significance of meatal plugs is widely appreciated, the molecular mechanisms that give rise to operable plugs are not yet understood. We fortuitously discovered comparable plugs

in C. elegans through our studies of FBN-1, which is a fibrillin-like apical matrix protein needed for worms to molt. The prevailing model for the process of molting includes 3 sequential steps: detachment of the epidermis from the preexisting cuticle; synthesis of the ensuing cuticle directly beneath it; and escape from the effete cuticle. As this model does not evoke an interim enclosure, it cannot explain how worms endure the process, rather than implode. We show that epidermal cells and syncytia actually produce a unified interim enclosure that bridges the effete and emergent cuticles. Therein, FBN-1 localizes both to exquisite plugs that occlude the bodily orifices and circumferential cables that corset the body. The FBN-1 rich mouth plugs evidently prevent mechanical separation of facial and pharyngeal epithelial across the molt. Additional findings suggest that FBN-1 molecules interact with two distinct pools of INA-1/alpha-integrin receptors embedded either in cellular or extracorporeal membranes. These dynamic interactions may enable the productive distribution and/or dissipation of forces at both cell and organismal levels. Going further, we identified NOAH-1 and -2, two additional Zona pellucida domain proteins, as auxiliary components of the larval sheaths using genetic and biochemical approaches. Our results provide an improved model for the very process of molting in which temporary atresia and corsetry are a vital first step. Findings about the interim sheaths of molting worms may apply to temporary appendages in mammals and related congenital birth defects.

P-262 MIG-6/papilin, an extracellular matrix protein, mediates the maintenance of neuronal architecture

Claire Bénard[1, 2], Malika Nadour[1], Lise Rivolet[1], Andrea Thackeray[2]

[1]*Université du Québec à Montréal*

[2]*University of Massachusetts Medical School*

After the initial assembly of the nervous system during embryogenesis, neuronal structures need to persist throughout life for neural circuits to remain functional, in the face of maturation, growth and body movements. How the nervous system is maintained lifelong remains to be understood. Our research using C. elegans has demonstrated that there are molecular mechanisms dedicated to actively maintaining the architecture of the nervous system. In neuronal maintenance mutants that we have identified, neuronal structures initially develop normally, but subsequently become disorganized. Neuronal maintenance genes act with great cell specificity. Here we report our identification, through forward genetic screens, of the gene mig-6/papilin as a novel neuronal maintenance factor. Loss of function of mig-6/papilin completely suppresses the progressive disorganization of sax-7/L1CAM mutants. Through whole genome sequencing, rescue assays and RNAi, we have uncovered the gene mig-6/papilin as a novel key player in neuronal maintenance. Using RNAi, we showed that mig-6 functions post-embryonically. To understand the mechanism of action of mig-6/papilin, we are addressing its expression pattern and site of action, and carrying out genetic and molecular analyses of mig-6 and its interactions other neuronal maintenance molecules and components of the extracellular matrix. We expect to uncover general principles of the maintenance of neuronal architecture, which may help to understand the basis of neurodegenerative conditions.

P-264 Shredding 3' UTRs

Jonathan Froehlich, Bora Uyar, Margareta Herzog, Altuna Akalin, Nikolaus Rajewsky

Berlin Institute for Medical Systems Biology, Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany

We present our approach to create many genetic variants for a locus of interest in parallel, which can be used to study coding and non-coding regions in C. elegans. We use this system to study regulatory elements with a focus on 3' UTRs. Using inducible Cas9, pools of sgRNAs and staged embryos we generate populations of worms with hundreds of different indel mutations for a locus of interest. After selecting animals for a phenotype or changed expression pattern we perform targeted sequencing using 0.5-3 kb amplicons to analyze indels. As a proof of concept and to establish our protocol we targeted non-coding regions of endogenously GFP-tagged histone H3.3 variant his-72. This ubiquitously expressed reporter allowed us to quantify and sort mutants with altered expression levels. We also hand-picked several mutants with abnormal expression patterns. We covered the native lin-41 3' UTR with different sets of sgRNAs. To understand if, besides the target sites of the miRNA let-7, there are other regions

required for viability, we are analyzing the depletion of genotypes over five consecutive generations of worms. We are currently also performing targeted RNA sequencing to determine the effects of different genotypes on RNA levels. We also mutated the 3'UTR of several genes (dpy-2, dpy-10, sqt-3, unc-26, unc-54, egl-30, snb-1) and isolated dozens of worms with different phenotypes. We aim to systematically cover these 3' UTRs with deletions to map regions with phenotypes. Furthermore, we can separate single mutants to quantify phenotypes and to characterize underlying mechanisms.

P-266 C. elegans early embryonic cytokinesis requires myosin motor activity

Joana Saramago[1, 2], Daniel Osório[1, 2], Fung Yi Chan[1, 2], et al.

[1]i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal.

[2]IBMC - Instituto de Biologia Molecular e Celular, Porto, Portugal.

Myosin is essential for cytokinesis but the importance of its motor activity has been controversial. In this study, we use C. elegans myosin motor mutants to define the role of non-muscle myosin II motor activity during early embryonic cytokinesis. We show that expression of motor-dead myosin leads to cytokinesis failure. Co-expression of motor-dead and wild-type myosin allows for completion of cytokinesis but slows it down without affecting actin levels in the ring. Embryos expressing partially motor-impaired myosin complete cytokinesis more slowly than controls and show enhanced sensitivity to a decrease in overall myosin levels. In the presence of motor dead myosin cortical actin flows are very reduced and erratic and do not define contractile ring formation. We propose that the contractile ring formation is due to the recruitment of sufficient motor-competent myosin to the cell equator from a cytoplasmic pool. Our results suggest that it is myosin motor activity that drives contractile ring assembly and constriction.

P-268 Proteostasis Regulation and Interplay

Carina I. Holmberg, Elisa Mikkonen, Sweta Jha

Research Programs Unit, Translational Cancer Biology, University of Helsinki, Helsinki, Finland.

Our group is studying regulatory mechanisms of proteostasis, in particular the ubiquitin-proteasome system (UPS) and its interplay with the autophagy-lysosome pathway (ALP). We have previously shown that proteasome activity is regulated in a tissue- and age-specific manner in C. elegans. Our recent results reveal that long-lived daf-2 mutants display similar proteasome tissue expression as aged-matched wild-type animals (1), although they exhibit higher proteasome activity in vivo and in vitro. We also demonstrate that a widespread proteasomal stress condition induced by RNAi elicits tissue-specific proteasome expression responses. In support of an interplay between UPS and ALP, our preliminary results show that modylating autophagy by knockdown of genes at different steps in this pathway may affect either proteasome activity alone in a tissue-specific manner or both proteasome activity and expression. A better understanding of tissue-specific regulatory mechanisms of proteostasis at the organismal level will aid translational research on aging and proteostasis-related diseases. 1. Mikkonen E, Haglund C, Holmberg CI. Immunohistochemical analysis reveals variations in proteasome tissue expression in C. elegans. PLoS One, 2017, 12:e0183403.

P-270 Computational Modeling of Early C. elegans Embryogenesis

Lidia Yamamoto, Rob Jelier

CMPG -KU Leuven

C. elegans embryogenesis has been intensively studied, however many underlying mechanisms driving the movements of the cells remain to be elucidated: How do cells find their correct positions in the embryo? How can they compensate mispositionings (Jelier et al. 2016)? What are the contributions of mechanical features such as the constrained space and cell-to-cell adhesion, and active movements, driven by actomyosin contractility? These questions remain unanswered even at the very early stages of development. We approach these questions via computational modeling and systematic imaging. Related modeling efforts (Fickentscher et al. (2013,2016), Wang

et al. (2016)) do not resolve the mechanisms sufficiently to answer the questions and test hypotheses. Therefore we developed a new simulator of early C. elegans embryogenesis, that explicitly models the physical interactions among cells that trigger cell motion, including cell and egg repulsion, adhesion, and active forces. The simulated cell nuclei positions are compared against a dataset of several wild-type embryos, where the cellular positions were measured over development by confocal imaging of GFP tagged nuclei and semi-automated tracking. Simulations with different parameter sets are evaluated based on a simulation error computed as the Procrustes distance between simulated and real nuclei positions. Within tested hypotheses, simulation parameters are optimized using evolutionary algorithms, minimizing the simulation error. Our system can identify actively moving cells and (differential) adhesion. Our results support a basal weak adhesion force among all cells, consistent with previous microscopy observations. Further, movements due to spatial constriction, divisions and adhesion forces are insufficient to explain the observed cell positioning even at the 8-cell stage. At this stage, ABpl and ABpr likely move actively to ensure the correct configuration of the embryo, with little evidence of differential adhesion between the cells.

P-272 CREB mediates a developmental plasticity in *Caenorhabditis elegans*

Jisoo Park[1], Yongjin Chun[1], Sundong Pyo[1], Scott J. Neal[2], Rebecca A. Butcher[3], Piali Sengupta[4], Kyuhyung Kim[1]
[1]*Department of Brain and Cognitive Sciences, DGIST, Daegu, 42988, Republic of Korea*
[2]*Department of Biochemistry, Yonsei University, Seoul, Republic of Korea*
[3]*Department of Ophthalmology, SUNY Upstate Medical University, Syracuse, NY 13210, USA*
[4]*Department of Chemistry, University of Florida, Gainesville, FL32611, USA*
[5]*Department of Biology and National Center for Behavioral Genomics, Brandeis University, Waltham, MA02454, USA*

Animals adapt to ever-changing environmental conditions via modulating developmental programs. Early stage of C. elegans larvae assess changes of environmental cues including food, temperature and levels of dauer-inducing pheromones as a crowding indicator, and decide either to proceed to reproductive developmental stage or to enter into the alternative, developmentally arrested dauer stage by regulating two parallel DAF-7 transforming growth factor-β (TGF-β) and DAF-2 insulin-like signaling pathways. Specifically, dauer formation is preceded by pre-dauer (L2d) decision of the first L1 larvae but the molecules and mechanisms underlying the L2d formation are elusive. Here, we show a novel role of crh-1 cAMP response element binding protein (CREB) in pre-dauer (L2d) decision. We found that crh-1 mutants inappropriately form transient L2d and express a L2d marker gene in non-dauer inducing condition with plenty of food and high concentrations of dauer pheromones. The L2d formation of crh-1 mutants is induced by ascr#5 but not by ascr#2 or ascr#3 pheromones, and crh-1 acts in the ascr#5 sensing ASI neurons to regulate ascr#5-mediated L2d formation. suggesting that crh-1 mediates the ascr#5 signal transduction. Moreover, the L2d formation of crh-1 mutants are mediated by DAF-7 TGF-β. daf-7 expression in ASI is down-regulated in crh-1 mutants, and promoter regions of the daf-7 gene contains a putative cyclic AMP- response element (CRE) site of which mutations decreased daf-7 expression, suggesting that CRH-1 directly regulates expression of daf-7 in the ASI. We also found that cmk-1 CaMKIV, akt-1 Akt/PKB, aak-2 AMPKs, and crtc-1 CRTCs regulate ascr#5-mediated L2d formation in parallel or downstream to crh-1. Taken together, these results provide new insight into how animals altered their development programs in response to changes in environments via transcriptional regulation of TGF-β with CREB.

P-274 Evaluation of nanoparticles in vivo using the Caenorhabditis elegans model organism

Anna Laromaine, Laura Gonzalez-Moragas, Luo Zhongrui, Anna Roig
Institut de Ciència de Materials de Barcelona, ICMAB-CSIC. Campus UAB. 08193 Bellaterra, Barcelona - Spain.

Nanoparticles (NPs) are present in many products and cosmetics, and are used in the food and medical industries. Tight regulations apply to the use of mammalian animals for product testing, hampering the study of the specific interactions between engineered nanoparticles and biological systems to better understand their potential effects. Here, we present the evaluation of 11-nm and 150-nm citrate-capped gold NPs and iron oxide nanoparticles (SPIONs) in the model organism C. elegans at multiple biological scales, moving from micrometric to nanometric resolution and from the organismal to subcellular levels. We confirmed that the nanoparticles were not able to cross the intestinal and dermal barriers of C. elegans. We investigated the effect of NPs on the survival and reproductive

performance of C. elegans, and correlated these effects with the uptake of NPs in terms of number, surface area, and metal mass. Current state-of-the-art techniques such as absorbance micro-spectroscopy, quantitative PCR of selected molecular markers and FTIR Synchrotron radiation to effects of NPs were applied.

P-276 Mechanical sensing of damage?

Rémy PUJOL[1], Chantal CAZEVIEILLE[1], Clara ESSMANN[2], David HALL[3], Jonathan EWBANK[4], Nathalie PUJOL[4]

[1]Institute for Neurosciences of Montpellier, Université Montpellier, INSERM, Montpellier, France.

[2]UCL TouchLab, Department of Computer Science, University College London, Engineering Building, Malet Place, London, WC1E 7JG, UK

[3]Center for C. elegans Anatomy, Albert Einstein Col Med, Bronx, NY 10461

[4]Centre d'Immunologie de Marseille-Luminy, AMU, INSERM, CNRS, Marseille, France

The cuticle, tightly linked to the epidermis, forms a physical barrier that protects worms from external injury and insults. Breaching this barrier, including during pathogen invasion, triggers a protective innate immune response in the epidermis. The cuticle is a complex structure composed of different collagens and with circumferential annuli and furrows regularly spaced along the length of the animal. A new cuticle is formed at each moult in a process involving sequential expression of collagen genes, including a subset of dpy genes expressed early and encoding collagens found uniquely in furrows (*dpy-2, 3, 7, 8, 9* and *10*). Interestingly, the corresponding furrow-less mutants exhibit a constitutive activation of immune, osmotic, and detoxification responses. We hypothesise that these divergent transcriptional stress responses are dependent upon a common sensor in the epidermis, potentially activated by mechanical stress and involving the furrow collagens (Dodd et al, Genetics 2018). To investigate this possibility, we have probed the surface of furrow-less *dpy-7* mutants with atomic force microscopy, revealing an altered stiffness. By transmission electron microscopy, we observed that the mutant cuticle does not have its usual 3-layered structure, missing the chevron-like layer, with abnormal struts and hemi-desmosomes. Initial results suggest that the mutants also have an alteration in an organelle found specifically in the epidermis. These structures are composed of 4-10 parallel plasma membrane folds, forming saucer-like stacks, variably external or internal to the plasma membrane, in contact with the cuticle and often associated with a mitochondrion. They had been presumed to be involved in cuticle deposition (White WormBook I, Politi et al IWM09). Interestingly, they line up in between furrows during moulting, when actin cables are found underneath the furrows in the epidermis. We are currently addressing the possibility that they could play a role in damage sensing, coupling cuticle tension to the epidermis.

P-278 Regulation of anterior fates by pop-1 and sys-1 in the C. elegans embryo

Jonathan Rumley[1], Amanda Zacharias[1, 2], John Murray[1]

[1]Department of Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA

[2]Division of Developmental Biology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA

In C. elegans, as in other animals, the Wnt pathway patterns the anterior-posterior axis, and its role in activating posterior-specific gene expression through the transcription factor (TF) POP-1/TCF and its coactivator SYS-1/β-catenin is well-characterized. However, the specification of anterior lineage fates is comparatively poorly understood. Wnt signaling can indirectly repress targets in posterior cells by activating expression of transcriptional repressors. Recent work, however, suggests TCF can also regulate these genes by direct binding. Through TCF either binding with additional TFs or binding to variant motifs, TCF can activate expression in the absence of signaling. Similar mechanisms may also allow TCF:β-catenin to repress expression in signaled cells. However, the relative importance of direct vs indirect mechanisms is not known. We used the EPIC database of embryonic expression patterns to identify over fifteen TFs expressed in anterior lineage-specific patterns. We used confocal imaging and automated lineage tracing software to compare the expression patterns of five of these TFs between untreated embryos and embryos with either pop-1 or sys-1 knocked down by RNAi. These experiments showed that each of these genes depends on pop-1 for activation in anterior cells or for repression in posterior cells, indicating they are

direct or indirect targets. Two of these genes also require sys-1 for repression in posterior cells. We have defined several short genomic sequences that can drive anterior-specific expression in enhancer assays and are currently dissecting these enhancers to define the mechanisms controlling their anterior-specific expression. As most of the anterior-specific TFs we are studying are essential, we are also testing their role in specifying anterior lineage identities by 4D imaging of mutants. Through this work, we will begin to learn how anterior fates are specified in the C. elegans embryo.

P-280 Netrin acts redundantly with the conserved homeobox gene *caudal*/*pal-1* to specify posterior patterning and axon guidance

Sophie Gilbert, Alison Woollard
University of Oxford

The homeobox gene *caudal* has been well studied for its highly conserved role in posterior body patterning. In *C. elegans*, null mutants of *caudal*/*pal-1* that are able to hatch do so without a posterior body portion (displaying a '*nob*' phenotype) and arrest development. From our own and previous observations, *pal-1* appears to be expressed in embryonic cells undergoing morphogenesis. Taking advantage of a rare, viable loss-of-function allele, *e2091*, we performed an RNAi screen of known regulators of cell movement and morphogenesis to look for genetic interactors of *pal-1*. From this screen, we have identified two sets of genes which interact with *pal-1*: members of the netrin pathway, and members of the wnt signalling pathway. Although wnt signalling has previously been shown to act with *caudal* both in *C. elegans* and in other organisms, a functional interaction between *caudal* and netrin has not been previously observed. Here we show that netrin and *pal-1* act redundantly to specify the posterior body plan of the embryo, with double mutants displaying a synthetic *nob* phenotype at hatching that phenocopies *pal-1* null alleles. This netrin-dependent morphogenesis in the posterior embryo is dependent on both UNC-5 and UNC-40 receptors. Other synthetic phenotypes include embryonic lethality, abnormal development of the male tail and larval axon migration defects, however, we have have found no role for *pal-1* in gonad migration. Our findings reveal a potentially conserved role for netrin in specifying global body patterning through direction of morphogenesis in the posterior embryo, as well as a new role for *pal-1* in specifying axon migration later in development.

P-282 Behavioural Screening to Predict Pesticide and Drug Mode of Action in C. elegans

Adam McDermott-Rouse, Andre Brown
Imperial College London

Finding and identifying the mode of action (MoA) of a drug often takes many months of work but offers a plethora of useful information, once found. The MoA gives us the identity of the pathways and systems the drug will affect and using this information can uncover the potential toxicity, specificity and likelihood of resistance for the specific drug in question. These traits will influence many aspects of its development and clinical/field use depending on its application. We hypothesise that drugs that share a MoA will illicit similar behavioural responses in individuals, while drugs that show dissimilar characteristics to the known responses may have a novel MoA. To test this, we created a database of behavioural fingerprints in C. elegans exposed to anthelmintic drugs relating to their posture and locomotion characteristics. This database includes 14 broad MoA classes and 30 subclasses of anthelmintics and was conducted over three concentrations (1, 10 and 100 µM). Using this database, we applied hierarchical clustering to group together the behavioural features based on Euclidean distance for each drug. We find several well separated clusters including vAChT inhibitors (spiroindoline type), associated with a coiling phenotype and GluCl agonists (mectin), which have reduced length and low curvature. However, there is not complete separation of all the classes. To improve this, we are going to increase the drugs in the library to better define the MoA classes. Also, some MoA classes have particular behavioural features that they associate with, therefore it may be useful to factor these into the classification.

P-284 The microRNA let-7 controls three distinct developmental events exclusively through the RNA-binding protein LIN41 and its four targets

Florian Aeschimann, Magdalene Rausch, Helge Großhans

Friedrich Miescher Institute for Biomedical Research, Maulbeerstrasse 66, 4058 Basel, Switzerland

The miRNA let-7 is a highly conserved regulator of stem cell self-renewal and differentiation in animals. In C. elegans, let-7 represses self-renewal of the epidermal seam cells and promotes morphogenesis of male and female sexual organs. Although miRNAs are thought to silence many targets, we previously showed that the defects in vulval development of let-7 mutants are caused by dysregulation of a single key target, the conserved RNA-binding protein LIN41/TRIM71. Surprisingly, we now find that the other two let-7 mutant phenotypes, the defects in male tail and seam cell development, also depend on a single target, and that this key target is again LIN41. As LIN41 silences several mRNAs, we wondered if its targets would explain the various functions of LIN41 downstream of let-7. Using RNA-IP sequencing, we find that somatic LIN41 appears to bind only four mRNAs, lin-29A, mab-10, mab-3 and dmd-3. Moreover, silencing of these targets indeed allows LIN41 to antagonize all let-7 functions. Thus, LIN-29A, an EGR-related transcription factor, and MAB-10, its NAB-orthologous co-factor, are responsible for let-7-mediated control of both seam cell self-renewal and vulva development. By contrast, properly timed morphogenesis of the male tail relies on the DM-domain transcription factors DMD-3 and MAB-3. We conclude that let-7-mediated repression of LIN41 promotes an adult identity of the epidermis, the vulva and the male tail by activating two distinct transcriptional pathways. The LIN41 targets thus seem to control specific functions downstream of a generic let-7-LIN41 module that defines the concerted onset of developmental events for the transition to adulthood.

P-286 The metabolic action of organoselenium compounds in C.elegans

Caroline Quines[1], Flávia Pereira[1], Cristina Nogueira[2], Gilson Zeni[2], Daiana Avila[1]

[1]*Universidade Federal do Pampas*

[2]*Universidade Federal de Santa Maria*

Organoselenium compounds have many pharmacological actions such as antihypercholesterolemic and antihyperglycemic by modulating the glucose and cholesterol metabolism in rodents. However, the molecular mechanism of these molecules is hard to study as knock out rodents are expensive and difficult to maintain. Caenorhabditis elegans (C.elegans) genome shows a high level of conservation with the vertebrate genome, which makes C. elegans an ideal system for metabolic studies. Our preliminary study investigated the effect of three organoselenium compounds: diphenyl diselenide (PhSe)2, p-chlorodiphenyl diselenide (p-ClPhSe)2 and p'-methoxyl-diphenyl diselenide (OMePhSe)2 in C.elegans (wild type). First, we evaluated the effect of these compounds in the worms survival at different concentrations: 0.5, 1, 10, 50, 100, 500 and 1000µM to determine the LC50 based on the worms survival rate 24h after the exposure (30m min of treatment). Furthermore, we also investigated the pharmacological action of these compounds at the same concentrations in the glucose and triglycerides levels in worms. Moreover, based on the LC50, we choose the lower concentrations (0.5, 1, 10 and 50µM) to investigate the effect of these compounds against the increase in glucose levels induced by fructose (550mM) in worms. The LC50 of these compounds were: (PhSe)2,:149.5µM, (p-ClPhSe)2:81.42µM and (OMePhSe)2:66.60µM indicating that (OMePhSe)2 was the most toxic to the worms. In relation to metabolic parameters, only the treatment with (p-ClPhSe)2 was effective in decreasing the glucose and triglycerides levels, with could be associated with the anti-obesity properties of this compound. In addition, treatment with the (PhSe)2, (p-ClPhSe)2 and (OMePhSe)2 protected against the increase in the glucose levels induced by fructose, which could be associated with the insulin mimetic properties of these compounds. Based on that, we will further take advantage of mutant strains to understand the mechanism of action of these compounds particularly focusing on the insulin-like signaling pathway in worms.

P-288 Histone Acetylations and Transcription Facilitate De Novo Centromere Establishment

Jing Zhu, Kevin Chi Lok Cheng, Karen Wing Yee Yuen

School of Biological Sciences The University of Hong Kong

The centromere is the specialized chromatin region that directs chromosome segregation. The kinetochore assembles on the centromere, attaching chromosomes to microtubules in mitosis. The centromere position is usually maintained through cell cycles and generations. However, new centromeres, known as neocentromeres, can occasionally form on ectopic regions when the original centromere is inactivated or lost due to chromosomal rearrangements. Centromere repositioning can occur during evolution. Moreover, de novo centromeres can form on exogenously transformed DNA in cells, which then segregates faithfully as artificial chromosomes (ACs). How centromeres are maintained, inactivated and activated is unclear. A conserved histone H3 variant, CENP-A, epigenetically marks functional centromeres, interspersing with H3. Several histone modifications enriched at centromeres are required for centromere function, but their role in new centromere formation is less clear. Studying the mechanism of new centromere formation has been challenging because these events are difficult to detect immediately, requiring selection in previous studies. DNA injected into the Caenorhabditis elegans gonad can concatamerize to form artificial chromosomes (ACs), also known as extrachromosomal arrays (Ex), in embryos, which first undergo passive inheritance, but soon autonomously segregate within a few cell cycles, more rapidly and frequently than human ACs. Using this in vivo model, we injected LacO repeats DNA, visualized ACs by expressing GFP::LacI, and monitored equal AC segregation in real time, which represents functional centromere formation. Histone H3K9 and H4 acetylations are enriched on new ACs when compared to endogenous chromosomes. By fusing histone deacetylase HDA-1 to GFP::LacI, we tethered HDA-1 to ACs specifically, reducing AC histone acetylations, equal segregation frequency, and initial kinetochore protein CENP-A/HCP-3 and NDC-80 deposition, indicating that histone acetylations facilitate efficient centromere establishment. Similarly, inhibition of RNA polymerase II-mediated transcription also delays initial CENP-A/HCP-3 loading. Acetylated histones on chromatin and transcription can create an open chromatin environment, contributing to centromere establishment.

P-290 Characterization of rme-8, a newly identified regulator of protein homeostasis

Mirjam Ax, Joanna Maus, Anna S Besemer, Christian von Hilchen, Heike Huesmann, Andreas Kern, Christian Behl, Albrecht M Clement

Institute of Pathobiochemistry, University Medical Center of the Johannes Gutenberg-University, Mainz, Germany

The maintenance of protein homeostasis (proteostasis) is of vital importance for cellular function under steady-state conditions and is continuously challenged particularly upon exposure to acute or chronic insults. In a functional screen in C. elegans aiming to identify new components of the proteostasis network, the knockdown of the gene receptor-mediated endocytosis 8 (rme-8; human ortholog: DNAJC13) induced protein aggregation. Accumulation of aggregation-prone proteins, such as the amyloid-ß 42 (Aß42) peptide, a-synuclein, or mutant variants of Cu/Zn-superoxide dismutase (SOD1) were aggravated upon the knockdown of rme-8 in C. elegans and DNAJC13 in human cell lines. In knockdown experiments we demonstrated that rme-8 is a positive modulator of autophagy under steady-state conditions as well as under autophagy-induced conditions. The importance of rme-8/DNAJC13 in neuronal function is documented as mutant DNAJC13 variant (N855S) cause familial form of Parkinson's disease with Lewy body pathology. Our investigations showed an alteration of cholinergic neurotransmission in C. elegans under rme-8 knockdown conditions. In summary, we demonstrate a novel function of rme-8 in cellular homeostasis by modulating autophagy and neurotransmission.

P-292 Microbial crystal proteins are not a worm's best friend

Hala Fahs[1], Fathima Refai[1], Robert White[1], Giselle Cipriani[1], Stephan Kremb[1], Glenn Butterfoss[1], Mireille Kallassy[2], Fabio Piano[1], Kristin Gunsalus[1]

[1]*New York University - Abu Dhabi*

[2]*Universite Saint-Joseph - Lebanon*

The discovery and characterization of biologically active molecules and their modes of action remain a challenge. We are using small molecule and natural products to identify novel compounds that affect worm development and study their modes of action. We established a high-throughput automated platform for chemical and functional genomic screening that accommodates both cell-based and whole-organism assays. We are focusing on anti-cancer

and broad-spectrum anthelmintics using the free-living nematode models C. elegans and the distantly related P. pacificus. Given the short life cycle of the worm, our platform enables one person to screen 20,000 chemicals per week and perform one genome-wide RNAi screen every three weeks. We validated our approach in a pilot screen of an FDA-approved drug library, which confirmed the effects of known anthelmintics on C. elegans and/or P. pacificus and revealed novel anthelmintic compounds. We screened a library of ~32,000 small molecules, selected using a computational approach to predict bioavailability in nematodes and identified numerous candidate molecules that will be assayed for toxicity in mammalian cells. We have also screened a Bacillus thuringiensis (Bt) library of 300 uncharacterized strains isolated in Lebanon and the UAE. Bt is a spore-forming bacterium that synthesizes crystal inclusions, certain of which show species-specific toxicity against insects, nematodes (i.e. Cry5B), and cancer cells. Bt crystal toxins therefore constitute a promising alternative to chemical anthelmintics. We found 95 strains that hinder the development of worms, and among them 50 strains that act through a Cry5-independent mechanism. Tests in the plant root-knot nematode parasite Meloidogyne revealed 20 strains with variable severity effects. Virulence factors of these strains are being characterized by DNA sequencing combined with functional genomic assays to elucidate their mechanisms of action.

P-294 Characterization of a p150/DNC-1 mutant that uncouples dynactin's role in dynein recruitment from its role in dynein activation

Joana Duro[1, 2], Daniel Barbosa[1, 2], Reto Gassmann[1, 2]

[1]*Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal*

[2]*Instituto de Investigação e Inovação em Saúde (I3S), Universidade do Porto, Porto, Portugal*

C. elegans relies on the molecular motor cytoplasmic dynein 1 (dynein) for the transport of diverse cargo towards microtubule minus-ends and for exerting pulling forces on microtubules during cell division that separate centrosomes in prophase and position the bipolar spindle. The multi-protein complex dynactin is essential for dynein localization and for activation of the motor, but the underlying molecular mechanisms remain incompletely understood. Dynactin's p150/DNC-1 subunit possesses an N-terminal CAP-Gly domain with microtubule-binding activity and an adjacent coiled-coil (CC) region, sub-divided into CC1A and CC1B, which binds the dynein intermediate chain subunit through CC1B. Here, we address the role of this DNC-1 region in vivo by characterizing deletion mutants, generated by CRISPR/Cas9-mediated genome editing, using live imaging. Deletion of the CAP-Gly domain results in spindle positioning defects in the one-cell embryo but does not compromise viability. By contrast, additionally deleting CC1A prevents centrosome separation and is 100 % lethal, just like DNC-1 depletions. Interestingly, DNC-1 without CAP-Gly+CC1A supports normal dynein localization to the cell cortex, kinetochores, centrosomes, and the nuclear envelope, suggesting that this DNC-1 mutant uncouples dynein recruitment from dynein activation. Surprisingly, despite the DNC-1 null phenotype in the early embryo, the CAP-Gly+CC1A deletion mutant does not appreciably perturb the kinetics of dynein-dependent cargo transport in touch receptor neurons. Thus, our results suggest that DNC-1's CC1A region has a context-dependent role in controlling dynein motor activity. We are currently using protein binding assays in vitro to determine whether DNC-1's CC1A modulates the interaction between CC1B and dynein intermediate chain.

P-296 Investigating the role of the ARF GTPase arf-3 in regulating seam cell development and secretion

Aidan Walker, Alison Woollard

University of Oxford

We have recently identified the promoter region of arf-3, a small GTPase implicated in intracellular trafficking, as the tissue-specific driver of the seam cell marker scm::gfp (identified in an enhancer trap screen) commonly used as a fate marker of seam cells in the study of seam cell development. RNAi of arf-3 leads to variable seam cell numbers suggesting a possible failure in the regulation of seam cell divisions. Furthermore, a deletion mutant of arf-3, tm1877, causes embryonic lethality, with escapees dying as larvae with seam abnormalities. We found that a translational reporter of arf-3 is cytoplasmic with ARF-3::GFP/mCherry localised to distinct puncta within the seam cells. The number and size of these puncta increase prior to ecdysis throughout the moulting cycle. Furthermore, ARF-

3::mCherry colocalises with the golgi marker RAB-6::GFP and late endosomal marker RAB-7::GFP prior to ecdysis, suggesting a role for arf-3 in regulating trafficking between the golgi and late endosomes, and cuticle deposition. The seam cells are known to play an important secretory function, giving rise to the cuticle and alae, but the cell biology of this is not well understood. Our observations suggest arf-3 may act as an important regulator of this process.

P-298 Investigation of mechanism of ketamine-induced anti-depressant effects in C. elegans

Duygu Yücel

Erciyes University, Genome and Stem Cell Center (GenKok), Kayseri, Turkey

Ketamine which has been exploited as a fast-acting anti-depressant in recent years, has long been used as an anaesthetic agent. Ketamine works as an N-methyl-D-aspartate receptor (NMDAR) antagonist however anti-depressant effects of ketamine have been shown to be mediated in an NMDAR-independent manner in murine models. Although low-dose ketamine levels are used in the clinic to combat major depressive disorder, the toxic effects of ketamine are inevitable. The mechanism of action of ketamine-induced rapid and sustained antidepressant effects are not clear. Elucidation of ketamine's antidepressant mode of action will enable development of new drugs to bypass drawbacks of ketamine. We have found that ketamine modulates glycosaminoglycan (GAG) synthesis in C. elegans which may be involved in its anti-depressant effect. The biosynthesis of GAGs in mammalians and the nematode is highly conserved. Growing body of evidence suggest a link between GAGs and psychiatric disorders such as depression, bipolar disorders and schizophrenia through regulation of neurotransmission and synaptic plasticity. The molecular mechanism by which ketamine acts to induce anti-depressant effect is currently under investigation.

Friday, 15 June 2018 - Auditorium - 18:00 – 18:30

Keynote address

Worm Tales

John White

Emeritus Professor Laboratory of Cell and Molecular Biology University of Wisconsin Madison, Wisconsin, USA

Following the elucidation of the genetic code in the early 60s many of the leading figures who were involved in this work turned their attention to applying the techniques of molecular genetics developed in prokaryotes to the study of development and nervous system function in eukaryotes. Sydney Brenner chose the soil nematode Ceanorhabditis elegans as a potential model system because of its small size, few somatic cells and short generation time. He established a bold research initiative to study this organism at the MRC's Molecular Biology Laboratory in Cambridge. I will describe how this initiative evolved in the 70s and 80s.

Friday, 15 June 2018 - Auditorium - 18:00 – 18:30

Special Keynote lecture

Comprehensively understanding *C. elegans* part 17: Dauer Development

Paul W. Sternberg, Pei Shih, James Lee, Cynthia Chai, Han Wang, Heenam Park, Jonathan Liu, Sarah Cohen

Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, USA.

The cellular simplicity and invariance of *C. elegans* development suggests that it is possible to comprehensively understand our favorite worm. This talk will discuss the state of our field using dauer larvae as an example. As we are all aware, *C. elegans* has a defined cell lineage, neuronal connectome and fully

sequenced genome. However, we have neither a complete collection of knockout mutants, nor gene expression for all cells, nor a defined metabolome, nor genetic accessibility for all cells. These would be helpful in understanding many aspects of *C. elegans* biology, for example dauer development. We will discuss progress along some of these lines, and use dauer larva as an example of what we need and what we can learn from comprehensive analyses. We deeply profiled the transcriptomes of whole larvae at seven time points as they decided whether or not to enter dauer from the L2d stage and found 8,000 genes with significant differences in gene expression among the various time points. We previously reported the massive upregulation of neuropeptides as worms progress towards and into dauer. We have also identified a set of transcriptional regulators whose differential expression correlates with the dauer decision. Using conditions in which intact wild-type larvae have approximately an 0.5 probability of entering dauer, we can readily analyze genes that affect the decision probability. This has allowed us to identify transcription factors and neuropeptide genes involved in the decision. In parallel, we have started applying the cGAL bipartite expression system to interrogate interneurons involved in the dauer decision, a relatively untouched part of the nervous system. These studies not only identify new genes and cells involved in dauer development but also indicate its staggering complexity, hence the need for yet more comprehensive studies.

Saturday, 16 June 2018 - Auditorium - 09:00 – 11:40

Session 7: Cell fate II (Neuronal) & Behaviour

S7-01 Homeoboxes build the *C. elegans* nervous system

Olivert Hobert

Columbia University, Howard Hughes Medical Institute, New York, USA

The relative simplicity of the *C. elegans* nervous system allows us to envision the possibility of a comprehensive, nervous system-wide understanding how a nervous system is genetically specified, i.e. how neurons acquire their unique and diverse identities and how they assemble into functional circuitry. An important tool in these endeavors are the availability of molecular maps that define neuronal identities throughout the entire nervous system. I will describe here how we have been using these molecular maps to uncover scores of regulatory factors (called terminal selectors) that specify the unique identity of individual neurons types. Currently, terminal selectors have been identified for almost 90 of the 118 distinct neuron classes. One emerging theme is the preponderance of homeobox genes in specifying neuronal identity. I will discuss here our ongoing analysis of the homeobox builders of neuronal identity and show an intriguing association of homeobox gene expression and function with neuronal connectivity.

S7-02 Glia and pioneer neurons direct hierarchical circuit formation through non-canonical redundant pathways of axon guidance in *C. elegans*

Georgia Rapti, Shai Shaham

The Rockefeller University New York, USA

Neuronal-circuit assembly requires a complex array of cellular-molecular interactions. It begins with early neuronal processes extending over non-neuronal substrates, often of glial origin. The identities, properties and interactions of early axons and glia are not well understood. To determine neuron-glia interactions initiating circuit assembly, we study the nerve ring (NR), the *C. elegans* brain-like neuropil consisting of ~180 axons and enveloped by four astrocyte-like CEPsh glia. Using time-lapse embryonic imaging, genetics, protein-interaction, cell ablations and functional studies, we uncover early events of NR assembly. We show that the NR is populated by glial and neuronal processes, in an orderly manner. Glia initiate assembly by guiding pioneer and follower axons through distinct molecular cues. Pioneer neurons with unique properties cooperate with glia to guide follower axons. We characterized a network of guidance pathways and isolated a mutant that spares axon-outgrowth initiation but severely disrupts early axon guidance. This led to identification of two proteins regulating non-canonically guidance-cue trafficking, in glia and pioneer neurons. We identified a unique

strategy to study redundancies, which have typically plagued genetic analysis of circuit formation. Using this genetic strategy we screened for novel redundant axon-guidance genes. We isolated over 20 mutants, numerous of which appear to be in previously unknown genes. Moreover, our studies highlight pioneer roles of glia and neurons of specific identities. The molecular identities and the morphogenetic determinants of such pioneers are not well understood in any model system. We are currently exploring novel strategies and tools to study early differentiation-morphogenesis of glia and neurons pioneering the *C. elegans* circuit formation. Our studies uncovered an in vivo model system to study glia-neuron interactions during circuit assembly. The genes we identify have conserved vertebrate homologs while embryonic CEPsh glia are reminiscent of vertebrate counterparts. Our studies, therefore, may reveal conserved mechanisms promoting circuit assembly.

S7-03 Repurposing of the kinetochore machinery during neuronal development

Dhanya Cheerambathur[1, 2], Bram Prevo[2], Arshad Desai[2]

[1]*Wellcome Centre for Cell Biology, University of Edinburgh, Edinburgh, U.K.*

[2]*University of California, San Diego, California, U.S.A*

Neuronal morphogenesis is coupled to dramatic changes in the microtubule architecture. During neuronal development, a radial microtubule array transforms to two structurally and functionally distinct microtubule networks, one with uniform polarity in the axon and the other with mixed polarity in the dendrite. However, the molecular mechanisms behind this are poorly understood. Here we show that components of the kinetochore, the conserved multiprotein complex that links DNA to microtubules during chromosome segregation, is redeployed during neurogenesis. Specifically, the microtubule-coupling module at the kinetochore, the 10-subunit KMN (Knl-1/Mis-12/Ndc-80) complex, has essential post-mitotic functions during early stages of neuronal morphogenesis. In contrast to dividing cells, endogenously tagged components of the KMN are enriched in cytoplasmic microtubule arrays in post-mitotic neuronal precursors. This localization of KMN is exclusive to a neuronal precursor in the developing embryo, where they associate with the microtubules of a polarized neurite undergoing extension. To investigate the function of the KMN network in neurogenesis, we employed a recently developed means to degrade GFP fusions in a tissue-specific post-mitotic manner in *C. elegans* embryos. Post-mitotic degradation of KMN components in sensory neuronal precursors leads to defects in neuronal morphology and organization of the nervous system, as well as compromised sensory neuronal activity. To determine the source of the neuronal defects, we developed an in situ high temporal imaging assay that captures the early stages of neurite extension. KMN components are essential for the proper extension of the microtubule-enriched dendrites of the developing neurons and defects in dendritic extension results in malformed neurons and an impaired adult neuronal network. Overall, our results highlight an essential role for the kinetochore machinery in regulating the microtubule cytoskeleton during neurogenesis and provide a striking example of the repurposing of evolutionarily ancient and essential cellular machinery to a new context in metazoan development.

S7-04 Dissecting the contribution of microRNAs to nervous system development and function

Chiara Alberti, Jingkui Wang, Luisa Cochella

IMP – The Research Institute of Molecular Pathology, Vienna

Nervous systems execute complex functions, supported by an outstanding diversity of neuron types. Neurons can be classified based on a variety of different functional, morphological or molecular features. In the worm, 118 classes of neurons have been distinguished based on the combination of these different properties. This diversity is generated through gene regulatory mechanisms, which have been studied mainly at the transcriptional level. However, post-transcriptional regulation, primarily repression, is also able to diversify broader transcriptional programs and contribute to cellular complexity. In particular, microRNAs (miRNAs), a class of post-transcriptional repressors, have been proposed to contribute to neuronal diversification. Not only are miRNAs enriched in the nervous system, but also many of them are expressed with very high neuron-type

specificity. Moreover, a few miRNAs have been shown to act as neuronal diversifiers using different mechanisms. To generate a complete view on the roles of miRNAs in the nervous system, we aim to obtain a cellular-resolution map of miRNA expression in the nervous system of the worm. This will reveal which combinations of miRNAs correlate with specific neuron classes and guide further functional studies. To this end, we have recently developed mime-seq, an innovative sequencing approach to gain access to microRNomes from individual cell types, without the need for cell sorting. Using mime-seq, we have profiled 12 partially-overlapping groups of neurons defined by neurotransmitter identity, presence of specific transcription factors and morphological features. We use linear regression to deconvolve the expression patterns measured for these larger groups into the individual constituents. In combination with miRNA reporter analysis, this will allow us to uncover the miRNome of individual neuronal classes. We will present the first system-level description of miRNAs in a nervous system, giving insight into potential redundancies and combinatorial activities to which we did not have access so far.

S7-05 A direct glia-to-neuron cell fate switch ensures nimble manoeuvres during male mating

Laura Molina-García[1], Steven J. Cook[2], Byunghyuk Kim[2], Rachel Bonnington[1], Michele Sammut[1], Jack O'Shea[1], David J. Elliott[1], David H. Hall[2], Scott W. Emmons[2, 3], Arantza Barrios[1], Richard J. Poole[1]

[1]Department of Cell and Developmental Biology, University College London, London WC1E 6BT, UK

[2]Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461, USA

[3]Department of Genetics, Albert Einstein College of Medicine, Bronx, New York 10461, USA

The coordinated execution of innate, stereotyped sexual behaviours, such as courtship and mating, requires sexually dimorphic sensory-motor circuits that are genetically specified during development. Studies in *C. elegans*, in which the development and function of neural circuits can be interrogated with single cell resolution, have revealed two general developmental mechanisms underlying sexual dimorphism in the nervous system. The first involves the acquisition of sexually dimorphic features in sex-shared neurons during sexual maturation, which include changes in terminal gene expression [1-7]. The second mechanism involves the generation of sex-specific neurons [8-10]. This requires sex-specific cell death [11] or neurogenesis events resulting from extensive sex differences in the cell division patterns and neurodevelopmental programmes of post-embryonic cell lineages. Here we identify a third, novel way to generate sexual dimorphism in the nervous system. We find that during sexual maturation (L4 stage), a class of sex-shared glial cells acquires sexually dimorphic function by undergoing a direct glia-to-neuron transdifferentiation that results in the production of male-specific neurons. This plasticity is regulated cell-intrinsically by the sex-determination pathway. These previously unnoticed neurons, which we term PHDs, are putative proprioceptors that regulate male locomotion during specific steps of mating. One of these steps is a novel readjustment movement performed when intromission becomes difficult to achieve. Our results reveal sex-specific direct transdifferentiation as a novel mechanism for generating sex-specific neurons and also show the importance of proprioceptive feedback during the complex steps of mating for successful reproduction. 1. doi:10.1126/science.1221762. 2. doi:10.1016/j.cub.2014.09.032. 3. doi:10.1038/nature17977. 4. doi:10.7554/eLife.21166. 5. doi:10.1534/genetics.117.202127. 6. doi:10.1016/j.cub.2016.11.045. 7. doi:10.1016/j.cub.2018.01.002. 8. Sulston JE, Horvitz HR. Dev Biol 1977;56:110–56. 9. Sulston JE, Albertson DG, Thomson JN. Dev Biol 1980;78:542–76. 10. doi:10.1038/nature15700. 11. Sulston JE, Schierenberg E, White JG, Thomson JN. Dev Biol 1983;100:64–119.

S7-06 Regulation of Long-Term Behavioral Patterns and Individuality across Development

Shay Stern[1], Christoph Kirst[2], Cornelia I. Bargmann[1, 3]

[1]Lulu and Anthony Wang Laboratory of Neural Circuits and Behavior, The Rockefeller University, New York, NY 10065, USA

[2]2Center for Physics and Biology and Kavli Neural Systems Institute, The Rockefeller University, New York, NY

10065, USA
[3]*Chan Zuckerberg Initiative, Palo Alto, CA 94301, USA*
Animals generate complex patterns of behavior across life that can be modified over days, months, or years. Across these long timescales, individuals within the same population may show stereotyped behaviors, but also unique behaviors that distinguish them from each other, a property called individuality. While individuality in behavior is widespread across species, including humans, the underlying mechanisms that generate individual-to-individual behavioral variation remain largely unknown. We examined the contributions of developmental programs and individual variation to behavior by developing a new multi-camera imaging system to monitor the behavior of multiple individual *C. elegans* animals across development, from egg hatching to adulthood, spanning a full generation time. By using this imaging system, we discovered that while *C. elegans* animals have reproducible patterns of long-term behaviors, individuals within isogenic populations show consistent behavioral biases that persist across development and distinguish them from one another. Furthermore, we identified specific signaling pathways that regulate stage-specific behaviors, and can either increase or decrease the degree of non-genetic individuality across the population. Overall, this study opened a new window for dissecting mechanisms that generate and shape behavioral individuality, as well as for understanding the organization and regulation of behavior across developmental time-scales.

S7-07 trp-1 and trp-2 TRPC channels mediate proprioceptive regulation of *C. elegans* locomotion

Jihye Yeon[1], Jinmahn Kim[1], Doyoung Kim[1], Hyunmin Kim[2], Daewon Moon[2], Kyuhyung Kim[1]
[1]*Department of Brain and Cognitive Sciences, DGIST, Daegu, 42988, Korea*
[2]*Department of New Biology, DGIST, Daegu, 42988, Korea*
For coherent locomotive behaviors, animals require highly coordinated sensorimotor feedback system, which is mediated by proprioceptive neurons and proprioceptors. The molecular mechanisms by which sensorimotor coordination are modulated by proprioception are not yet clear. Here we show the TRPC channels trp-1 and trp-2 are important mediators of the proprioception that guides steering locomotion during forward movement in *C. elegans*. We found that trp-1 trp-2 double, but not single mutant animals make ventral-directed circles during forward movement. trp-1 and trp-2 are expressed in the SMDD proprioceptive neurons and the trp-1 trp-2 mutants show impaired SMDD calcium activity in response to head bending. Functional defects in SMDD and optogenetic manipulation of SMDD activity to mimic that observed in the trp-1 trp-2 mutants also cause ventral circling. Two proprioceptors, *C. elegans* trp-4 and Drosophila trpγ, can functionally substitute for trp-1 or trp-2 in locomotor behavior, and ectopic expression of TRP-1 or TPR-2 confers head bending-dependent responses on a *C. elegans* chemosensory neuron. Thus, the *C. elegans* TRPC channels trp-1 and trp-2 act as proprioceptors and coordinate the precise sensorimotor activities required for proper head movement.

S7-08 Embryonic Exposure to Amphetamine Reduces Gene Expression of the Dopamine Transporter

Lucia Carvelli[1], Ganesh Ambigapathy[2], Talus McCowan[2], Archana Dhasarathy[2]
[1]*Brain Institute, Harriet L. Wilkes Honors College - Florida Atlantic University*
[2]*Department of Biomedical Sciences - University of North Dakota, Grand Forks ND*
Despite the widespread use as psychostimulant and therapeutic drug, the long-term consequences of amphetamine (AMPH) have been poorly investigated. Among its other effects, AMPH alters the function of proteins uniquely associated with the reward system, i.e. the dopamine transporter (DAT). Similarly to mammals, *C. elegans* exhibits changes in behaviors when treated with AMPH, and we showed that these changes are largely mediated by the *C. elegans* DAT (DAT-1). Here we investigated the behavioral and functional effects caused by chronic AMPH exposure during embryogenesis in *C. elegans* adults and progeny. We found that animals that were pre-exposed to AMPH exhibited higher values of AMPH-induced behaviors during adulthood. Interestingly, this effect was inherited by the progeny. Because DAT-1 is one of the proteins required to generate AMPH-induced behaviors in both *C. elegans* and mammals, we tested whether embryonic

exposure to AMPH alters the landscape of histone methylation associated with the promoter of the dat-1 gene. Our ChIP experiments show that at the promoter of dat-1 of adult animals, embryonal AMPH exposure causes significant changes of specific histone markers associated with gene silencing. Interestingly, these same changes were observed also in progeny (F1 generation). In vitro experiments demonstrate that the ability of DAT-1 to reuptake dopamine was decreased in primary cultures of dopaminergic neurons (F1 generation). Taken together, these data suggest that chronic AMPH exposure during embryogenesis reduces expression of DAT-1 in adult animals and this reduction, not only causes behavioral effects, but it is also transmitted to progeny.

S7-09 Sexy learning in *C. elegans*: Integration of conflicting experiences

Laura Molina-Garcia, Sergio Benavides-Laconcha, Arantza Barrios
Department of Cell & Developmental Biology, University College London, UK

When an animal repeatedly encounters a signal coupled with either a punishment or a reward, it eventually learns to expect both to occur together in a process called associative learning. A central goal in neuroscience is to understand how neural circuits integrate conflicting (rewarding and aversive) experiences that need to be behaviourally resolved during learning. To shed light into this process at the molecular and cellular level, we are dissecting a neural circuit for sexual conditioning in the *C. elegans* male. Sexual conditioning is a form of male-specific associative learning by which a rewarding experience with mates overrides an aversive association with starvation, thus switching the males' behaviour to a stimulus from repulsion to attraction (Sakai et al., 2013) Previously, Sakai et al. (2013) and us (Sammut et al. (2015)) have shown that males undergo sexual conditioning to salt. Here we show that males can be sexually conditioned to other chemosensory stimuli and this requires the male-specific MCM interneurons and the neuropeptide PDF. Two models have been proposed for how conflicting memories may be integrated during learning but conclusive mechanistic evidence for either one is lacking (Aso & Rubin, 2016). In one model, each memory is processed in parallel with different decay rates. In the alternative model, memories are integrated through reciprocal inhibition. The connectivity of the MCMs to the circuit for chemotaxis learning could support either form of integration (Sammut et al., 2015). If integration occurs through reciprocal inhibition of reward and aversion, the prediction is that sexual conditioning will prevent the molecular changes that drive aversive learning (Cho et al.2016) from occurring. Here we show that some of the molecular changes underlying aversive learning still occur during sexual conditioning. This provides the first piece of evidence supporting model 1: both memories are formed and compete for behavioural expression.

Saturday, 16 June 2018 - Auditorium - 13:00 – 15:20

Session 8: Metabolism & Microbe-host interactions

S8-01 A persistence detector for transcriptional metabolic network rewiring in an animal

Albertha J.M. Walhout
Program in Systems Biology, University of Massachusetts Medical School, Worcester, MA, 01605, USA

Persistence detection is a mechanism that ensures that a physiological output is only executed when the relevant input is sustained. It has been proposed that a gene regulatory network (GRN) circuit known as a coherent type 1 feed-forward loop (FFL) with an AND-logic gate can generate persistence detection1. In such a circuit two transcription factors (TFs) are both required to activate a target gene and one of the two regulators (TF1) activates the other (TF2), resulting in a delay in target gene expression. While FFLs have been identified in several systems, examples of transcriptional persistence detectors have only been described for bacteria. In addition, they have been used in synthetic bacterial circuitry. However, their existence and utility in animal physiology has not been explored. I will present a transcriptional persistence detector in the nematode Caenorhabditis elegans. This persistence detector involves the nuclear hormone receptors (NHRs) NHR-10 and NHR-68 that together activate the expression of five propionate shunt pathway genes. This shunt is an

alternative propionate breakdown pathway that is used only when flux through the canonical, vitamin B12-dependent propionate breakdown pathway is perturbed. The first step in the propionate shunt pathway generates acrylyl-CoA, which can be interconverted with acrylate, a highly toxic metabolite. The propionate persistence detector only activates the shunt pathway when propionate accumulates for a prolonged amount of time, and, therefore, prevents shunt activation when only transient spikes of propionate occur. We propose that the propionate persistence detector functions to avoid spurious production of toxic acrylate such that propionate is preferentially catabolized through the canonical pathway. This work demonstrates that transcriptional persistence detectors can be used to ensure physiological homeostasis in an animal.

S8-02 Neurohormonal signalling via a cytosolic sulfotransferase controls insulin sensitivity of *C. elegans*

Nick Burton[1, 2], Vivek Dwivedi[2], Kirk Burkhart[2], Rebecca Kaplan[3], L. Ryan Baugh[3], H. Robert Horvitz[2]

[1]*Centre for Trophoblast Research, Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, CB2 1HP, UK*

[2]*Howard Hughes Medical Institute, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA*

[3]*Department of Biology, Duke University, Durham, NC 27708, USA*

Insulin and insulin-like growth factor signalling regulates a broad spectrum of growth and metabolic responses to a variety of internal and environmental stimuli. Such responses can be tailored so that changes in insulin signalling result in distinct physiological responses to different stimuli. For example, the inhibition of insulin-like signalling is key in the responses of the nematode *C. elegans* to both osmotic stress and starvation, but these two stresses result in responses that are both physiologically and molecularly distinct. How does reduced insulin-like signalling elicit different responses to different environmental stimuli? We report that neurohormonal signalling involving the *C. elegans* cytosolic sulfotransferase SSU-1 controls developmental arrest in response to osmotic stress but does not control the distinct developmental arrest that occurs in response to starvation. SSU-1 functions in a single pair of sensory neurons to control intercellular signalling -- likely by catalyzing the synthesis of a steroid hormone -- via the nuclear hormone receptor NHR-1. SSU-1-controlled signalling antagonizes insulin-like signalling and hence modulates insulin sensitivity. In short, we describe a previously unknown neurohormonal signalling pathway that is required specifically for some but not all consequences of reduced insulin-like signalling. In mammals, the nervous system plays a similarly important yet poorly understood role in modulating insulin sensitivity. Our results suggest that the mammalian nervous system might regulate insulin sensitivity via sulfotransferase-controlled neurohormonal signalling.

S8-03 WormJam: A consensus *C. elegans* Metabolic Reconstruction and Metabolomics Community

Janna Hastings[1], Nicolas LeNovere[1], Michael Witting[2], Olivia Casanueva[1]

[1]*1Epigenetics Department, Babraham Institute, Cambridge CB223AT, United Kingdom*

[2]*Analytical BioGeoChemistry, Helmholtz Zentrum, München, Germany*

Metabolism has increasingly been recognised as an important contributor to *C. elegans* healthspan and lifespan, as well as forming the basis of adaptation to different environments. Most of the historical efforts to detect changes in metabolism in *C. elegans* have been based on molecular tools including gene expression and/or reporter strains. However, more recently, systems biology metabolic modelling approaches developed in single-cell organisms have started to be applied to multicellular organisms as well, and several reconstructions of the whole-genome metabolism of *C. elegans* were independently developed in the past three years. WormJam (short for Worm Jamboree) has been established as a platform for the community effort of reconciling the existing *C. elegans* metabolic reconstructions and expanding the unified consensus reconstruction through targeted annotation jamborees. The availability of a community-driven consensus reconstruction of *C. elegans* metabolism lays the foundation for bringing *C. elegans* to the forefront of

metabolism research. In our lab, we are using the WormJam consensus reconstruction to predict how metabolic fluxes change during ageing using Flux Balance Analysis together with a multi-omics time-series dataset.

S8-04 Host-Environment Interactions: Metabolic cross-talk for Ageing and Cancer

Filipe Cabreiro
University College London

In the past century, attempts to understand human disease were focused on identifying mutations in the genome responsible or associated with a disease state. Despite great advances in our understanding of disease from this genomic-centric approach, we still do not fully comprehend why similar mutations can lead to a wide-range of disease manifestations. Recent evidence shows that disease states arise from the complex interactions between the genetic make-up of the host and its environment. Nutrition and the microbiome are key environmental factors regulating host physiology but studying these in the context of drug efficacy remains a great challenge. Combining two tractable genetic models, the bacterium E. coli and the nematode *C. elegans*, we are currently unravelling the complexity underlying such interactions in the efficacy of fluoropyrimidine anticancer drugs 1 and the anti-diabetic drug metformin 2,3. Currently, using a 4-way drug-microbe-nutrient-host high-throughput screening approach combined with multi-omics at the host and microbe level (the holobiont) we find that the microbiota integrates nutrition and drug cues through complex signalling networks to drive unique phenotypical outputs in the host. Health benefits to the host conferred by the impact of drugs on the microbiota can be recapitulated through targeted genetic manipulation of signalling or metabolic pathways in bacteria. Importantly, genetic or pharmacological interventions targeting the microbiota improve host health in other animal models such as Drosophila and mice. Overall, our data shows that the mechanistic understanding of the effects of diet, drugs and intestinal microbiota on host physiology allows their manipulation and may improve health in humans.

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S8-05 Rapid recruitment of non-centrosomal microtubules is required for immune activation after wounding

Clara TAFFONI, Shizue OMI, Caroline HUBER, Jolanta POLANOWSKA, Jonathan EWBANK, Nathalie PUJOL
Centre d'Immunologie de Marseille-Luminy, AMU, INSERM, CNRS, Marseille, France

Repair of skin wounds and activation of immune responses are essential for fighting infection. How these responses are initiated and coordinated remains poorly understood. In the epidermis of *C. elegans*, wounding provokes an immediate calcium burst that promotes actin ring formation at the wound site, enabling wound closure, and an increase in the transcription of antimicrobial peptide (AMP) genes (Pujol et al 2008 CB; Xu et al 2011 CB). To decipher the cell biology of this response, we wound the adult syncytial epidermis with a 405nm laser under a spinning disk microscope. After the calcium burst, we observe rapid membrane reorganization at the wound site, similar to membrane patching (Davenport et al 2016 MboC), followed by a highly polarized recruitment of EB1/EBP-2, a protein that binds the + end of microtubules. This precedes the formation of the actin ring necessary for wound closure, a process distinct from the classic contractile purse string machinery. At the same time, the SLC6 bioamine transporter SNF-12, which is a key player in the epidermal immune

response (Dierking et al 2011 CHM), gets locally activated. We propose a model where upon wounding, SNF-12 dissociates from apical clusters, gets cleaved and the released C-terminal domain translocates into the nucleus to activate the innate immune response, associating with the STAT-like transcription factor STA-2. Inactivation of specific isoforms of microtubules disrupts the normal localization of SNF-12 and its activation upon wounding, leading to an absence of a subsequent immune response. Thus, in addition to revealing an unexpected activation of a bioamine transporter, our work suggests that cytoskeleton reorganization during wound closure is closely linked to immune activation.

S8-06 Characterization of the starvation survival response mediated by the elongation factor kinase efk-1/eEF2K

Forum Bhanshali[1], Andy An[1], Jennifer Watts[2], Asad Jan[1], Poul Sorensen[1], Stefan Taubert[1]
[1]*University of British Columbia (UBC)*
[2]*Washington State University.*

In its natural environment, *C. elegans* is likely to frequently encounter nutrient-limiting conditions. To survive prolonged periods of starvation, *C. elegans* has developed starvation-survival strategies enabling it to persist until conditions become favorable for growth, which include downregulation of energy-consuming processes such as protein synthesis. The elongation factor kinase eEF2K is an evolutionarily conserved serine/threonine kinase that negatively regulates protein synthesis by inactivating the translation elongation factor eEF2, which protects cells from acute nutrient deprivation. In *C. elegans*, the eEF2K ortholog efk-1 is transcriptionally upregulated by starvation and hypoxia and is required for larval starvation survival and normal lifespan. However, the pathways that induce efk-1 during starvation as well as downstream efk-1-regulated targets and processes that enable starvation survival are unknown. To delineate efk-1 induction, we quantified efk-1 mRNA expression in fed and starved wild-type worms and in worms carrying mutations in various transcription factors (TFs) known to regulate starvation responses. We found that the TFs hlh-30/TFEB and daf-16/FOXO are required to upregulate efk-1 mRNA upon starvation. To characterize how efk-1 promotes starvation survival, we performed GC-MS analysis and found that efk-1 mutants were unable to catabolize TAG efficiently in starvation suggesting the potential role of efk-1 in lipid metabolism. Next, we explored parallels between the starvation response and the response to pathogen toxins, which induce translational arrest by inhibiting EEF-2. In these scenarios, translation arrest activates downstream transcriptional programs driven by the TFs ZIP-2 and CEBP-2. Interestingly, we found that zip-2 and cebp-2 mutants are sensitive to starvation, so they may act downstream of efk-1 to promote starvation survival. To unbiasedly identify which pathways are modulated by efk-1 under starvation, we are performing RNA sequencing on fed and starved WT, efk-1, zip-2 and cebp-2 mutants. Overall our study has uncovered the new players in EFK-1 stress response pathway for the control of mRNA translation elongation and survival in nutrient scarce conditions.

S8-07 You are what you experience: The impact of environment on cellular identity

Sarah Becker, Marie-Charlotte Morin, Séverine Mangold, Sophie Jarriault
IGBMC

The balance between maintenance of cellular identity and cellular plasticity (as the potential of identity change on a functional and morphological level) is a major challenge for tissues inside an organism. Unbalanced, uncontrolled cell fate changes can cause several dysfunctional cellular behaviors such as cancer and degenerative diseases. Unraveling the mechanisms behind cell type conversion will help to develop a safe environment for regenerative medicine. Here, we describe how several external factors can impact on cellular identity and increase its potential of plasticity. We use a natural cell identity conversion in the worm to determine how a cell can change or maintain its identity. *C. elegans* rectal to neuronal Y-to-PDA transition is a bona fide transdifferentiation event: During L2 larval stage the epithelial identity of the Y rectal cell is erased completely, followed by a very robust and unipotent redifferentiation into a fully functional motoneuron, PDA. We previously described a subset of essential factors that are crucial for the inititation of Td such as egl-

27/MTA, sem-4/SALL, ceh-6/OCT and sox-2, whose loss of function lead to severe defects in PDA formation. We identified two novel regulators in Td: lin-15A and lin-56. Their null mutants show a lower penetrance of PDA defects and are highly variable under different environmental conditions. We found starvation and caloric restriction, as well as virulence or different food sources to decrease PDA defects in these mutants and thus to increase the potential of cellular plasticity. We have indications that there is a general mechanism underlying the effect of these environmental factors and that this might be a more general concept not only in the worm, that could impact the field of cell identity conversions as a tool in regenerative medicine.

Saturday, 16 June 2018 - Auditorium - 13:00 – 15:20

Session 9: DNA repair & cell death

S9-01 DNA damage responses in aging and disease: lessons from the worm

Björn Schumacher

Institute for Genome Stability in Aging and Disease, Medical Faculty, Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD) Research Centre and Centre for Molecular Medicine (CMMC), University of Cologne, Joseph-Stelzmann-Str. 26, 50931 Cologne, Germany

The causal contribution of DNA damage in driving the aging process has become evident in a variety of progeroid syndromes that are caused by defects in DNA repair systems. Congenital defects in genome maintenance mechanisms cause complex disease phenotypes characterized by developmental growth failure, cancer susceptibility, and premature aging. The distinct human disease outcomes of DNA repair defects are particularly apparent in syndromes caused by mutations in nucleotide excision repair (NER). While transcription-coupled (TC-) NER defects lead to growth and mental retardation and premature ageing in Cockayne syndrome (CS) patients, global-genome (GG-) NER mutations lead to highly skin cancer prone Xeroderma pigmentosum (XP). Intriguingly, the distinct outcomes of NER deficiencies are conserved in the simple metazoan *C. elegans*. TC-NER deficiency renders worms highly susceptible to DNA damage during developmental growth and with aging, while GG-NER defects give rise to genome instability in proliferating germ cells.

We employed the nematode model to investigate distinct DNA damage response (DDR) mechanisms in (postmitotic) somatic tissues and in the germline. DNA damage that persists in somatic tissues leads to activation of the insulin-like growth factor signalling (IIS) effector DAF-16. The FoxO transcription factor DAF-16 is efficiently activated in response to DNA damage during development while its DNA damage responsiveness declines with aging. We demonstrated that DAF-16 alleviates growth arrest and enhances DNA damage resistance in somatic tissues even in the absence of DNA repair. We propose that IIS mediates DNA damage responses in somatic tissues and that DAF-16 activity enables developmental growth amid persistent DNA lesions and promotes maintenance of differentiated tissues through enhanced tolerance of DNA damage that accumulates with aging. Mechanistically, we determined that the conserved ERK1/2 MAPK pathway regulates the DAF-16-mediated DDR. An integrated proteomics, phospoproteomics and lipidomics analysis of the in vivo response to persistent UV-induced DNA lesions revealed a comprehensive picture of the organism's DDR. We determined a shift in proteostasis towards autophagy, a dampening of glucose and lipid metabolism, and functionally implicate IIS, EGF-, and AMPK-like signalling. We found striking similarities between the acute response to DNA damage and the proteome of aging animals thus further supporting the underlying role of DNA damage accumulation in the aging process. Our data provide new insights into the organism's response program to DNA damage during development and aging and suggest new intervention targets for triggering stress responses to antagonize the detrimental consequences of genome instability.

S9-02 EFF-1 fusogen promotes phagosome sealing during cell process clearance

Piya Ghose[1], Alina Rashid[1], Peter Insley[1], Anupriya Singhal[1], Pavak Shah[2], Yun Lu[1], Zhirong Bao[2], Shai Shaham[1]
[1]*Laboratory of Developmental Genetics, The Rockefeller University, New York, NY, USA.*
[2]*Developmental Biology Program, Sloan Kettering Institute, New York, NY, USA.*

Programmed cell death and cell process pruning are common in development and homeostasis. Dismantling of morphologically complex cells, with long processes, poses a particularly interesting problem, as different regions of a cell are often in different microenvironments, and contact different cells. We study this in the *C. elegans* tail-spike cell (TSC). The TSC extends a process during embryonic morphogenesis and then dies. TSC ablation suggests that the cell functions in tail morphogenesis. Still images and long-term light-sheet microscopy reveal that the TSC undergoes three distinct degenerative events. The proximal TSC process is dismantled first, and undergoes Wallerian degeneration-like beading. The cell soma then dies and is cleared similar to other apoptotic cells. The distal process retracts and accumulates in a distinct varicosity. All three mechanisms depend independently on CED-3/caspase. Importantly, a similar sequence accompanies the demise of embryonic and sexually dimorphic CEM neurons. Thus, the TSC death program may represent a general mechanism for dismantling complex cells, including neurons. Clearance of the process is independent of known apoptotic engulfment proteins, suggesting novel mechanisms. From a genetic screen, we identified a mutant with a lesion in eff-1, encoding a fusogen, which is defective in TSC distal process varicosity clearance specifically. EFF-1 is expressed in and functions in the engulfing cell for the TSC process. While the remnant is recognized in eff-1 mutants, the phagosome remains open. FRAP, an open phagosome reporter and electron micrographs support this. EFF-1's fusogenic function is required for clearance. EFF-1 also localizes to phagosome arms tips. Thus, EFF-1 may mediate the fusion event required for phagosome sealing. Direct mediators of membrane scission that promote phagosomes formation are not known. Our data reveal a novel paradigm for complex cell dismantling that may be broadly conserved, and suggest a novel role for EFF-1 in phagosome sealing.

S9-03 Maintenance of genome integrity by Mi2

Carolyn Turcotte, Solomon Sloat, Julia Rigothi, Erika Rosenkranse, Alexandra Northrup, Nicolas Andrews, Paula Checchi
Marist College

Meiotic recombination depends upon the tightly coordinated regulation of chromosome dynamics and is essential for the production of haploid gametes. Central to this process is the formation and repair of meiotic double-stranded breaks (DSBs), which must take place within the constraints of a specialized chromatin architecture. Here, we demonstrate a role for the nucleosome remodeling and deacetylase (NuRD) complex in orchestrating meiotic chromosome dynamics in *C. elegans*. Our data reveal that the conserved Mi2 homologs Chromodomain helicase DNA binding protein (CHD-3) and its paralog LET- 418 facilitate meiotic progression by ensuring faithful repair of DSBs through homologous recombination. We discovered that loss of either CHD-3 or LET-418 results in elevated p53-dependent germline apoptosis, which relies on the activation of the conserved checkpoint kinase CHK-1. Consistent with these findings, chd-3 and let-418 mutants produce a reduced number of offspring, indicating a role for Mi2 in forming viable gametes. When Mi2 function is compromised, persisting recombination intermediates are detected in late pachytene nuclei, indicating a failure in the timely repair of DSBs. Intriguingly, our data indicate that in Mi2 mutant germ lines, a subset of DSBs are repaired by non-homologous end joining, which manifests as chromosomal fusions. We find that meiotic defects are exacerbated in Mi2 mutants lacking CKU-80, as evidenced by increased recombination intermediates, corpses, and defects in chromosomal integrity. We are currently generating several strains which will enable us to further understand the molecular nature of these defects and will report on our findings, which thus far support a model wherein the Mi2 complex maintains genomic integrity through reinforcement of a chromatin landscape suitable for homology-driven repair mechanisms.

S9-04 Genome-wide RNAi screen in *C. elegans* identifies compromised mitochondrial protein import as a signal for the induction of UPRmt

Stephane Rolland[1], Sandra Schneid[1], Melanie Schwarz[1], Elisabeth Rackles[1], Christian Fischer[1], Simon Hauessler[1], Saroj Regmi[1, 3], Assa Yeroslaviz[4], Bianca Habermann[4, 5], Eric Lambie[1], Barbara Conradt[1, 2]

[1]*Department Biology II, Faculty of Biology, Ludwig-Maximilians-University Munich, 82152 Planegg-Martinsried, Germany*

[2]*Center for Integrated Protein Science, Ludwig-Maximilians-University Munich*

[3]*Division of Molecular and Cellular Biology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA.*

[4]*Max Planck Institute for Biochemistry, Computational Systems Biochemistry, Am Klopferspitz 18, 82152 Martinsried*

[5]*Aix Marseille Univ, CNRS, IBDM, Marseille, France*

The induction of the 'mitochondrial unfolded protein response' (UPRmt) results in increased transcription of the gene encoding the mitochondrial chaperone HSP70. We systematically screened the *C. elegans* genome and identified 172 genes that when inactivated induce the expression of an hsp-6 HSP70 reporter and encode mitochondrial proteins. These genes represent many but not all mitochondrial processes. For example, we found that defects in mitochondrial calcium homeostasis or mitophagy fail to induce UPRmt. Those mitochondrial processes that do induce UPRmt when compromised do so in a manner that is dependent on the transcription factor ATFS-1 but not the kinase GCN-2. In addition, directly or indirectly, these processes are required for the maintenance of membrane potential across the inner mitochondrial membrane and, hence, for robust protein import into mitochondria. Finally, we propose that compromised mitochondrial protein import can act as a signal for the induction of UPRmt and present evidence that the mitochondrial targeting sequence of ATFS-1 functions as a sensor for this signal.

S9-05 The cisd gene family regulates physiological germline apoptosis through ced-13 and the canonical cell death pathway in Caenorhabditis elegans

Skylar King[1], Chipo Gray[1], Luhua Song[1], Rachel Nechushtai[2], Tina Gumienny[3], Ron Mittler[1], Pamela Padilla[1]

[1]*Department of Biological Sciences, University of North Texas, Denton, TX 76203*

[2]*Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, Edmond J. Safra Campus at Givat Ram, Jerusalem 91904, Israel*

[3]*Department of Biology, Texas Woman's University, Denton, TX 76204*

Programmed cell death, which occurs through a conserved core molecular pathway, is important for fundamental developmental and homeostatic processes. The human iron-sulfur binding CISD protein NAF-1/CISD2 binds to Bcl-2 and its disruption in cells leads to an increase in apoptosis. Other members of the CISD family include mitoNEET/CISD1 and Miner2/CISD3. Disruption of CISD1 and CISD2 in mammalian cell culture leads to various phenotypes associated with mitochondrial dysfunction and cell proliferation defects. In humans, mutations in CISD2 result in Wolfram syndrome 2, a disease in which the patients display juvenile diabetes, neuropsychiatric disorders and defective platelet aggregation. The *C. elegans* genome contains three previously uncharacterized cisd genes that code for CISD-1, which has homology to mitoNEET/CISD1 and NAF-1/CISD2, and CISD-3.1 and CISD-3.2, both of which have homology to Miner2/CISD3. Disrupting the function of the cisd genes resulted in a significant increase in the number of cell corpses within the adult germline. This increased germ cell death is blocked by a gain-of-function mutation of the Bcl-2 homolog CED-9 and requires functional caspase CED-3 and CED-4. Furthermore, the increased germ cell death is facilitated by the pro-apoptotic, CED-9-binding protein CED-13, but not the related EGL-1 protein. This work is significant because it places the cisd gene family members as regulators of physiological germline programmed cell death acting through CED-13 and the core apoptotic machinery. Furthermore, it is the first study to show function for a CISD3 protein family member.

Sunday, 17 June 2018 - Auditorium - 09:00 – 11:35

Session 10: Ageing

S10-01 Small nucleoli are a cellular hallmark of longevity

Adam Antebi

Max Planck Institute for Biology of Ageing, Cologne, Germany

Research in model organisms over the last several decades has revealed that animal lifespan is plastic and regulated by conserved metabolic signaling pathways, which work through specific transcription factors to extend life. Whether these pathways affect common downstream mechanisms remains largely elusive. Our studies demonstrate that NCL-1/TRIM2/Brat tumor suppressor extends lifespan and limits nucleolar size in the major *C. elegans* longevity pathways, as part of a convergent mechanism focused on the nucleolus. Animals representing distinct longevity pathways exhibit small nucleoli, and decreased expression of rRNA, ribosomal proteins, and the nucleolar protein fibrillarin, dependent on NCL-1. Fibrillarin is not only a marker but itself is a causal factor whose knockdown reduces nucleolar size and extends lifespan. Long-lived dietary restricted fruit flies and insulin-like-peptide mutants also exhibit reduced nucleoli and fibrillarin expression. Similarly, tissues derived from long-lived dietary restricted and reduced insulin/IGF signaling IRS1 knockout mice, and humans who undergo modest dietary restriction coupled with exercise display reduced nucleoli. We suggest that small nucleoli are a cellular hallmark of longevity and metabolic health conserved across taxa.

S10-02 Endosomal/autophagic regulation of the DAF-16 transcription factor

Icten Meras, Laëtitia Chotard, Christian E. Rocheleau

Departments of Medicine and Anatomy and Cell Biology, McGill University, Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada, H4A 3J1

FOXO proteins are a conserved family of transcription factors that regulate metabolism, stress responses, and aging. In *C. elegans*, DAF-16 the sole homolog of FOXO, is negatively regulated by Insulin/IGF-like Signaling pathway (IIS). Signaling from DAF-2, the receptor of the (IIS) pathway, activates the AKT1/2 kinase, which phosphorylates DAF-16. The 14-3-3/FTT-2 proteins bind phosopho-DAF-16, sequestering it in the cytoplasm. We found that DAF-16 localizes to a subset of RAB-5 and RAB-7 positive endosomes in intestinal cells of *C. elegans*. In starved animals, DAF-16 endosomes are lost, where it mainly localizes to the nucleus, whereas re-feeding results in relocalization of DAF-16 onto endosomal membranes. Futhermore, loss of daf-18, a negative regulator of IIS, results in an increase in the number of DAF-16 endosomes, while knockdown of akt-1, but not akt-2, results in loss of DAF-16 endosomal localization where it accumulates mainly in the nucleus. We found that FTT-2/14-3-3 proteins colocalizes with DAF-16 on endosomes and knockdown of 14-3-3 protein, FTT-2, results in loss of DAF-16 endosomes. This may be conserved in human cells as our preliminary data indicate that insulin treatment promotes pFOXO1/3a localization to RAB5 positive endosomes in HEK293 cells. We do not know the ultimate fate of pFOXO/DAF-16 on endosomes, but our data shows that LC3/Atg8 engulfs DAF-16 endosomes and knockdown of LC3/Atg-8 increased the number of DAF-16 endosomes, suggesting that they might be degraded by selective autophagy. We hypothesize that IIS pathway regulates FOXO proteins on endosomes where it can be released if needed or degraded via selective autophagy. Relevance: Here we are proposing a new mechanism of FOXO regulation on signaling endosomes and turnover by selective autophagy. We believe this project will provide new insights into the regulation of FOXO proteins.

S10-03 TORC2 regulates the maturation of endosome via SGK-1 in the intestine of *C. elegans*

Yijian Yan[1], Wenjing Qi[1], Ralf Baumeister[1, 2]

[1]*Bioinformatics and Molecular Genetics (Faculty of Biology), Albert-Ludwigs-University of Freiburg, Freiburg, Germany*

[2]*ZBMZ (Faculty of Medicine), Albert-Ludwigs-University of Freiburg, Freiburg, Germany*

SGK-1, the only Caenorhabditis elegans homolog of hSGKs, acts in parallel to the AKT kinases to mediate DAF-2 signaling to regulate metabolism, growth, development, and longevity. Besides, SGK-1 is the direct downstream target of the TORC2 kinase Rictor/RICT-1. In addition, SGK-1 is the downstream component of the cold-sensitive TRPA-1 calcium channel and calcium-sensitive PKC-2 in a signaling pathway that detects temperature reduction. Thus, SGK-1 integrates several pathways in the regulation of metabolism, development, longevity, and stress response in *C. elegans*. Beyond its role in the crosstalk between insulin signaling and TORC2 pathway, hSGK3 was recently reported to localize to endosomes and render cancer cells resistant to prolonged treatment by PI3K or Akt inhibitors. Like hSGK3, structural predictions indicate that *C. elegans* SGK-1 also contains a Phox homology domain that is thought to preferentially bind to phosphatidylinositol-3-phosphate, therefore enabling its endosomal localization in a VPS-34 dependent manner. We now show that SGK-1 may also be a regulator of endosome/lysosome system in polarized epithelial cells of *C. elegans* intestine. Misexpression of sgk-1 results in vacuole-like structures (VLSs) in intestinal cells. Co-localization analysis with various markers demonstrated that VLSs are enlarged endosomes in which cargos of the endocytic pathway were retained. Besides, sgk-1 mutant was reported to missort cargo proteins against the default destinations, which additionally supports its function in endomembrane trafficking. We further showed that sgk-1 mutant contain significantly increased lysosome-related organelles (LROs), which was suggested to be originated from Golgi network and early-late endosome, strengthened the role of SGK-1 in endocytic pathway. Epistatic analysis showed that the endosomal function of SGK-1 was dominantly mediated by TORC2 pathway and VPS-34 complex, while insulin/DAF-2 and TRPA-1 pathways do not play functional roles. This suggests that serum-and-glucorcorticoid-inducible kinases act in a conserved VPS34-TORC2 pathway involved in endocytic trafficking and signaling transduction.

S10-04 In vivo luminescent ATP *C. elegans* sensor strains for drug discovery in age-related diseases – an update.

Cristina Lagido

Institute of Medical Sciences, The School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Aberdeen AB25 2ZD, UK

Targeting of mitochondria holds promise for treatment of multiple diseases. Here, an ATP biosensor *C. elegans* that expresses firefly luciferase constitutively and reports on cellular ATP in vivo by luminescence (strains PE255 and PE254; Lagido et al. 2008, 2015; McLaggan et al. 2012), was crossed into genetic models of neurodegenerative disease. A proof-of-principle drug screen for mitochondrial toxicity and/or modulation was then carried out with the focus on pink-1, the *C. elegans* homologue of the PTEN-induced kinase 1. This mitochondrial enzyme is involved in mitochondrial quality control and linked to human familial early onset Parkinsonism. The NINDS (National Institute of Neurological Disorders and Stroke) compound library of 1040 drugs, 75% of which are FDA-approved, was tested at 10 uM initially, in the pink-1 (ok3538) genetic background. Compounds with direct effects on the activity of purified firefly luciferase in vitro were eliminated as false positives. 23 drugs were selected for testing of concentration responses in the pink-1 and wildtype genetic backgrounds. Data for eleven compounds that resulted in concentration dependent decline will be presented. The mitochondrial toxins rotenone, thimerosal, chlorpromazine and chlorothalonil pestanal displayed the strongest concentration-dependent effects, validating this approach. Additionally, four drugs caused an enhancement of response, three of them known mitochondrial modulators. A more pronounced decline in the pink biosensor strain luminescence was observed in response to drugs such as thimerosal, chlorothalonil pestanal and amiodarone, in agreement with reported sensitivity to oxidative stress of loss of PINK1 (Gautier et al. 2008). Phenotypic screening may pinpoint novel targets with potential to improve mitochondrial function in disease. This will also contribute to reduction of higher animal testing and of late phase failure of drug candidates. Clark et al. (2006) Nature 441, 1157-1161 Lagido et al. (2008) BMC Physiology 8(7) Lagido et al. (2015) JoVE(104), e53083 McLaggan et al.(2012) PLoS One 7(10):e46503.

S10-05 Modifiers of age-related protein aggregation and toxicity

Ellen Nollen

University of Groningen, University Medical Center Groningen, European Research Institute for the Biology of Aging, Groningen, The Netherlands

Toxic aggregation-prone disease proteins are thought to play a major role in the pathology of several age-related neurodegenerative disorders, including Alzheimer's and Parkinson's disease. We aim to uncover cellular pathways that regulate disease-protein toxicity and aggregation. With genome-wide genetic screens in *C. elegans* models for protein aggregation diseases, we have identified a variety of modifiers of proteotoxicity, which include MOAG-2/LIR-3 and MOAG-4/SERF. MOAG-2/LIR-3 normally regulates small non-coding RNA transcription in the nucleus. When aggregation-prone proteins are present it moves to the cytosol where it appears to catalyse aggregation. These results suggest that aggregation-prone proteins can hijack cellular proteins and use them to propagate their own aggregation and toxicity, providing a new mechanistic explanation for the progressive nature of age-related protein toxicity. MOAG-4 and its human counterparts SERF1 and SERF2 catalyse aggregation through a direct and transient interaction with disease proteins, in a way that has not been reported previously. SERF1 and SERF2 are highly abundant in many cell types and act on several amyloid-prone proteins, including those that cause major neurodegenerative diseases such as Alzheimer's and Parkinson's disease. We currently aim to i) establish how SERFs, and other modifiers drive proteotoxicity using genetic and biochemical approaches in human cells and worms, ii) uncover their physiological role in development and during aging in mouse models, and iii) screen for modifiers of protein toxicity in *C. elegans* models for protein misfolding diseases using newly developed automated high throughput tools. Our results will reveal biological mechanisms that drive early proteotoxic events in aging and age-related diseases and which are candidate targets for therapeutic inhibition.

S10-06 GSK-3 intestinal activity impacts mitochondrial function and ageing

Francisco Javier García-Rodríguez[1], Annmary Paul Erinjeri[1], Artur Bastos Lourenço[1], Mary Doherty[2], Phillip Whitfield[2], Peter Askjaer[1], Marta Artal-Sanz[1]

[1]*Andalusian Centre for Developmental Biology (CABD), CSIC-Universidad Pablo de Olavide, Seville, Spain*

[2]*Department of Diabetes and Cardiovascular Science, University of the Highlands and Islands, Inverness, UK*

Impaired mitochondrial function is a hallmark of ageing and age-related pathologies. The mitochondrial prohibitin complex, composed of PHB-1 and PHB-2, has emerged as a context-dependent modulator of lifespan. While PHB-1 or PHB-2 depletion shortens the lifespan of wild type worms, it dramatically extends lifespan under compromised metabolic conditions, as is the case of daf-2(e1370) mutants. To better understand the function of PHBs in ageing regulation, we performed a phenotype-based RNAi screening for prohibitin regulatory kinases. We identified the Glycogen Synthase Kinase -3 (GSK-3) as a suppressor of the reduced Nile Red phenotype in both phb-2(tm2998) and phb-2(tm2998);daf-2(e1370) mutants. GSK-3 is the worm orthologue of human GSK-3β, a pleiotropic kinase involved in multiple cellular processes and linked to different diseases such as Alzheimer's, diabetes, cancer or neurodegeneration. Interestingly, GSK-3 depletion decreases the lifespan of wild-type worms and strongly suppresses the long-lived phenotypes of daf-2 and daf-2;phb-2, while it mildly affects phb-2 mutants. We used CRISPR/Cas9 to generate a gsk-3 endogenous gene tagging. GSK-3 is ubiquitously expressed and shows different cellular localization patterns depending on the tissue and prohibitin expression. We combine transcriptomic analysis and metabolic assays to demonstrate a role for GSK-3 as a global metabolic regulator. GSK-3 alters glycogen and fat stores and impacts mitochondrial lipid composition and respiration in a genotype-dependent manner. Moreover, we identify a gsk-3-dependent transcriptional blueprint related to ageing where intestinal specific genes are overrepresented. By SapTrap toolkit, we generated different gsk-3 tissue-specific endogenous knockouts and demonstrate that GSK-3 impact on ageing specifically relies on its intestinal activity.

S10-07 UNC-120/SRF independently controls muscle aging and lifespan in Caenorhabditis elegans

Florence Solari, Adeline Mergoud dit Lamarche, Laurent Molin, Laura Pierson, Marie-Christine Mariol, Kathrin Gieseler, Jean-Louis Bessereau

NeuroMyoGeneInstitute, CNRS UMR5310, INSERM U1217,University of Lyon

Aging is commonly defined as the loss of global homeostasis, which results from progressive alteration of all organs function. This model is currently challenged by recent data showing that interventions that extend lifespan do not always increase the overall fitness of the organism. These data suggest the existence of tissue-specific factors that regulate the pace of aging in a cell-autonomous manner. Here, we investigated aging of Caenorhabditis elegans striated muscles at the subcellular and the physiological level. Our data show that muscle aging is characterized by a dramatic decrease in the expression of genes encoding proteins required for muscle contraction, followed by a change in mitochondria morphology, and an increase in autophagosome number. Myofilaments, however, remain unaffected during aging. We demonstrated that the conserved transcription factor UNC-120/SRF regulates muscle aging biomarkers. Interestingly, the role of UNC-120/SRF in the control of muscle aging can be dissociated from its broader effect on lifespan. In daf-2/insulin/IGF1 receptor mutants, which exhibit a delayed appearance of muscle aging biomarkers and are long-lived, disruption of unc-120 accelerates muscle aging but does not suppress the lifespan phenotype of daf-2 mutant. Conversely, unc-120 overexpression delays muscle aging but does not increase lifespan. Overall, we demonstrate that UNC-120/SRF controls the pace of muscle aging in a cell-autonomous manner downstream of the insulin/IGF1 receptor.

S10-08 Assessing involvement of the four C. elegans ACADSB orthologues in (healthy) lifespan and metabolism

Brecht Wouters[1], Clara Verschuuren[3], Ellen Geens[2], Winnok H. De Vos[4], Bart P. Braeckman[3], Ineke Dhondt[3], Liliane Schoofs[1], Liesbet Temmerman[2]

[1]Laboratory for Functional Genomics and Proteomics, Department of Biology, KU Leuven, Leuven, Belgium

[2]Laboratory of Molecular and Functional Neurobiology, Department of Biology, KU Leuven, Leuven, Belgium

[3]Laboratory for Aging Physiology and Molecular Evolution, Biology Department, Ghent University, Ghent, Belgium

[4]Laboratory of Cell Biology and Histology, Department of Veterinary Sciences, University of Antwerp, Antwerp, Belgium

Like any other animal, humans are subjected to the process of ageing. As we grow older, it becomes increasingly difficult to maintain homeostasis and deal with the implications of a deteriorating body, raising the incidence of many age-related diseases including cancer, neurodegenerative diseases, sarcopenia, osteoporosis and metabolic syndrome. Impaired nutrient sensing, causative to metabolic syndrome and cancer, is considered to be a primary hallmark of ageing. Many metabolic pathways have long been acknowledged as regulators of the ageing process, which include pathways such as the insulin signaling and mTOR pathways. Degradation of branched-chain amino acids (BCAAs) and lipids are hypothesized to be altered in metabolic syndrome. One gene sparks our interest, namely the short/branched chain specific acyl-CoA dehydrogenase (ACADSB) gene, as it influences both processes. ACADSB is involved in the degradation of the branched-chain amino acid isoleucine and short/branched chain acyl-Coa derivatives in the beta oxidation pathway. The ACADSB gene is conserved over several taxa, including the model organism C. elegans. We studied the four ACADSB orthologues in C. elegans via RNAi knockdown. We performed lifespan experiments, evaluating the effect of tissue-specific knockdown of these genes on lifespan using two different bacterial diets. Additionally, integrity of the muscles was checked in old day 14 worms as a marker for healthy lifespan. To assess the effect on lipid metabolism, fat staining and quantification was performed. These initial experiments have proven to be invaluable in understanding the role of each ACADSB orthologue gene in C. elegans, while opening perspectives for further metabolomic studies.

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Acknowledgement of Sponsorship

The organizing committee gratefully acknowledges the generous support of the following organizations.