Global insights in life sciences: bridging European and Japanese research

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Abstract book

Keynote speakers

Molecular basis of astrocyte-neuron interactions in synapse regulation

Yukiko Goda
Okinawa Institute of Science and Technology Graduate University

Synapses are key mediators of information transmission in the brain. The ease with which synaptic transmission occurs, termed synaptic strength, undergoes dynamic changes that play crucial roles in neural circuit operations underlying cognitive processes. Recent studies have highlighted the contribution of astrocyte network in shaping synaptic circuit activity with consequences on behavior in variety of brain regions. Yet, precisely how astrocytes interact with synapses and how they modify individual synaptic strengths, especially in local circuits, remains to be fully understood. We have sought to clarify the cellular organization and the molecular basis that shape tripartite synapses in the hippocampus by focusing on the one hand on cell adhesion proteins and on the other hand on diffusible signaling. Our recent findings will be presented.

Tau clustering and dynamics of Neuronal Na^+/K^+-ATPase

Antoine Triller
Institut de Biologie de l'Ecole Normale Supérieure (IBENS)

Proteins such as α-synuclein, amyloid-β, tau and SOD1 whose aggregation is associated to neurodegenerative diseases directly bind and impair α3-Na^+/K^+-ATPase activity. Neuronal Na^+/K^+-ATPase is responsible for the maintenance of ionic gradient across plasma membrane. The α3-subunit containing Na^+/K^+-ATPase expression is restricted to neurons. Heterozygous mutations within α3-subunit leads to rapid-onset dystonia parkinsonism, alternating hemiplegia of childhood and other neurological and neuropsychiatric disorders.
I will present recent and yet unpublished results, on fTau, mTau time- and concentration- dependent clustering on the neuronal plasma membrane and its control by a Na^+/K^+-ATPase derived peptide. The diffusion properties of mTau and fTau on the membrane indicate that tau clustering depends on lateral diffusion, and that trapping within tau clusters exacerbates over time. The formation and maintenance of tau clusters on the neuronal membrane is therefore the result of a dynamic process. Furthermore, we demonstrate that a 27-amino acid peptide derived from an extracellular loop of the α3-Na^+/K^+-ATPase prevents tau accumulation on the membrane of cortical neurons in primary culture and cultured organotypic brain slices. This paves the way for a new therapeutic strategy aimed at delaying the progression of tauopathies.

Session I | Hematopoietic stem cell development and dysregulation in leukemia

Inflammatory signals in hematopoiesis

Hitoshi Takizawa
Kumamoto University

Hematopoiesis is a continuous blood production that is sustained by hematopoietic stem cells (HSCs), a somatic stem cell that lifelong selfrenew and differentiate into all blood and immune cells. When hematopoietic stress such as infection, inflammation, chemotherapy occur, HSC and progenitor function substantially change, i.e., attenuate HSC selfrenewal, bias their differentiation potential, etc. We have studied a role of innate immune signaling in HSCs and their offspring in the context of infection, inflammation and leukemogenesis, opening the field of stress hematopoiesis. More recently, we have found that microbial infiltration caused by tissue damage and natural aging, adapt hematopoietic program to enhance myelopoiesis, suggesting a crosstalk between bone marrow and other tissues which might serve as a tissue surveillance mechanism. Based on these findings, I will discuss regulatory mechanisms underlying development and maintenance of hemato-immune system throughout lifetime.

Presentation type: Invited speaker - regular talk

Modelling childhood leukemia using human IPS cells

Tariq Enver
Cancer Institute, University College London

Childhood acute lymphoblastic leukaemia is thought in many cases to initiate in utero. The factors that influence the formation of the initiating lesions are not well understood although the nature of these genetic aberrations is well documented and include in approximately a quarter of children the presence of the t12;21 chromosomal translocation that fuses the transcription factors Tel (ETV6) and Aml1 (RUNX1). The Etv6-Runx1 fusion gene produces a pre-leukaemic clone but in and of itself is insufficient to produce frank leukaemic transformation. For this, additional mutations are required and it remains unclear what factors influence their acquisition. To gain insight with these issues we have been exploring the target genes of Etv6-Runx1 and associated second hits as well as developing new foetal specific models in which to examine the biological impact of Etv6-Runx1. Our results allow us to develop

transcriptional networks that inform how different mutations may collaborate to allow leukemic progression. As part of this analysis we revealed that Etv6-Runx1 functions as a first hit mutation primarily through competition for RUNX1 binding sites and transcriptional repression. In frank leukemia, knockdown of RUNX1 or its co-factor CBFβ results in cell death suggesting sustained requirement for RUNX1 activity which is recapitulated by chemical perturbation using an allosteric CBFβ-inhibitor. Strikingly, we show that RUNX1 addiction extends to other genetic subtypes of paediatric B-ALL and also adult disease. Importantly, inhibition of RUNX1 activity spares normal hematopoiesis. Our results suggest that chemical intervention in the RUNX1 program may provide a therapeutic opportunity in ALL. Finally, modelling of the independent and combinatorial role of mutations associated with ALL gives insight into the mechanisms underlying leukaemogenesis in this disease.

Presentation type: Invited speaker - regular talk

Regulation of hematopoietic stem cell dormancy

Nina Cabezas-Wallscheid
Max Planck Institute of Immunobiology and Epigenetics and ETH Zürich

Hematopoietic Stem Cells (HSCs) rely on complex metabolic and epigenetic regulatory networks to preserve their function. Due to the scarcity of HSCs, technical challenges have limited our insights into the interplay between HSC metabolism and their transcriptional and epigenetic regulation. We recently have established new low-input multi-layer OMICs methods to address the metabolic, lipidomic and epigenetic hubs that are enriched in mouse and human HSCs and their downstream progenitors, upon aging and in the context of leukemia (Schönberger*, Obier* et al., Cell Stem Cell 2022; Lalioti*, Romero-Mulero* et al., revisions). Mechanistically, we uncover a non-classical retinoic acid signaling axis that regulates HSC identity. Our findings emphasize how a single metabolite controls stem cell fate by instructing epigenetic and transcriptional attributes. Now, we have used these knowledge to in vivo modulate HSC activity upon myocardial infraction, thus emergency hematopoiesis, to improve the heart function (Rettkowski*, Romero-Mulero* et al., accepted). Further, we have recently shown that the G-protein coupled receptor family C group 5 member C (GPRC5C) is a regulator of human HSC dormancy (Zhang et al., Nature Cell Biology 2022). High GPRC5C levels in AML correlate with poor survival and promote leukemia aggression via NF-κB activation and increased branched-chain amino acids (BCAAs; Zhang et al., Blood Advances 2023). Targeting the BCAA transporter SLC7A5 with JPH203 inhibits leukemia growth, sparing healthy bone marrow, and enhances the

effect of venetoclax and azacitidine. This suggests the GPRC5C-NF-κB-SLC7A5-BCAA axis as a therapeutic target in AML.

Presentation type: Invited speaker - regular talk

Normal progenitor turnover is a tumor suppressor in T and B cell development

Robin Thiele (1,2) , Verena Körber (3,4) , Csilla Kongsaysak-Lengyel (1) , Thorsten Feyerabend (1) , Thomas Höfer (3) , Hans-Reimer Rodewald (1)

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Cancer is generally thought to be initiated by oncogenic mutations. Yet, cancer driver mutations are found frequently also in normal tissues, leading to cell clones with selective advantages that, however, normally fail to progress to cancer. This discrepancy raises the important question which conditions predispose for malignant transformation. We demonstrated that the absence of cell competition among early T-cell progenitors in the thymus promotes at high frequencies the development of T-cell acute lymphoblastic leukemia (T-ALL) in mice. These T-ALL exhibit recurrent genomic lesions that mirror the human disease. This system lends itself to cellular and molecular studies into a natural transition from normal healthy cells to leukemia. We now provide evidence for a second model of tumorigenesis through a similar perturbation, in that limiting stem/progenitor contribution to the B-cell lineage leads to B-cell lymphomas. These transplantable B-cell lymphomas display recurrent genetic lesions, including chromosome 18 trisomy and a deletion in the Kmt2c locus. In conclusion, these experiments support the concept that, at least in hematological malignancies, stochastically acquired driver mutations become 'unleashed' for malignant transformation through extended progenitor dwell times.

Presentation type: Invited speaker - abstract talk

Session II | Biological role of sleep - from molecules to systems

REM sleep gating and dopamine signaling in mice

Emi Hasegawa [1], Ai Miyasaka [2], Katsuyasu Sakurai [2], Yoan Cherasse [2], Yulong Li [3], Takeshi Sakurai [2]

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The sleep cycle alternates between REM (rapid eye movement) and NREM (non-rapid movement) sleep, which is a highly characteristic feature of sleep. However, the mechanisms by which this cycle is generated are totally unknown. Neuroimaging and intracranial recording studies have shown amygdala activation during REM sleep in humans. We found that a periodic transient increase of dopamine (DA) level in the basolateral amygdala (BLA) during non-rapid eye movement (NREM) sleep terminates NREM sleep and initiates REM sleep. DA acts on dopamine receptor D2 (Drd2)-expressing neurons in the BLA to induce a transition from NREM to REM sleep. This mechanism also plays a role in cataplectic attack, which is a pathological intrusion of REM sleep into wakefulness in narcoleptics. In narcoleptic patients, cataplexy is often triggered by positive emotion and amygdala activity increases in cataplexy, which suggests roles of the amygdala and reward system in cataplexy. We found that DA levels in the BLA transiently increased before cataplexy attacks in narcoleptic mice but not in wild-type mice. Positive emotion might induce a transient increase of DA in the BLA in narcoleptics but not in wild types, mimicking the DA dynamics that trigger NREM-to-REM transitions. This is consistent with a study that showed an increase in DA levels in narcoleptic dogs only in the amygdala. We previously showed that the activity of serotonin (5-HT) neurons in the dorsal raphe, which are excited by orexin, is involved in the suppression of cataplexy, which suggests that this pathway might inhibit the release of DA in the BLA through 5-HT–mediated inhibition of DA axonal terminals. These results show a critical role of DA signaling in the amygdala in REM sleep regulation and provide a neuronal basis of sleep cycle generation.

Presentation type: Invited speaker - regular talk

A SW-REM CPG and its activation

Gilles Laurent
Max Planck Institute for Brain Research

I will summarize my lab's recent results concerning sleep dynamics in the Australian dragon Pogona vitticeps, a diurnal vertebrate whose biphasic sleep cycle is both very short (1.5-2mins) and extremely regular, yielding between 200 and 300 sleep cycles per night, each with a duty cycle of about 0.5. I will present evidence for the existence of a bilateral pair of weakly coupled central pattern generator circuits, of a type typically found to control motor systems, as responsible for this alternating SW-REM rhythmic activity. I will also show how these CPGs are turned on and off at the end and beginning of the day.

Presentation type: Invited speaker - regular talk

Molecular dissection of the two-process model of sleep regulation

Maria Robles
Institute of Medical Psychology and Biomedical Center, LMU

Sleep is a universal physiological state, conserved across the animal kingdom and essential for learning and memory, metabolic homeostasis, and immune function. Despite its critical importance, the functions of sleep remain largely unknown at the molecular and cellular levels.
More than 40 years ago, Alexander Borbély proposed the two-process model of sleep regulation, which describes two interconnected mechanisms underlying sleep modulation. These mechanisms are the sleep homeostat (also known as Process S), which measures the accumulation of sleep pressure as a function of time spent awake, and Process C, based on circadian function.
Our recent and ongoing work employs multi-omics techniques, with quantitative proteomics at their core, to study the molecular foundations of the two-process model of sleep regulation. Our aim is not only to molecularly dissect these two mechanisms in the whole brain but also to examine their contributions in diverse brain areas, specific cell type populations, and subcellular compartments.

Presentation type: Invited speaker - regular talk

Neuroplasticity during sleep

Gordon Feld

Central Institute of Mental Health

Sleep is crucial for memory processes. On the one hand sleep is important for consolidation on the other hand it promotes synaptic rescaling. I will introduce the two main theories on neuroplasticity during sleep. I will also briefly present two datasets (one behavioural one neuroscientific) from my work that have measured these processes.

Presentation type: Invited speaker - regular talk

Session III | Biological impact of ageing

Genetic program of age-dependent behavioral decline in C. elegans

Kentaro Noma

Nagoya University

Our neuronal functions decline with aging. Accumulation of DNA damage, oxidative stress, and diseases such as Alzheimer's have been proposed as mechanisms of age-dependent decline of neuronal functions. However, can these passive mechanisms explain the differences in aging speed among species? We hypothesize that the genetic program may actively cause neuronal aging. The major challenges to studying the aging of neuronal functions are the long aging time and the complexity of the nervous system. Therefore, we use C. elegans, which has a two-week lifespan and shows various behaviors with a simple nervous system. Many of these behaviors diminish after they cease reproduction. We conducted novel genetic screening and found a mutant that did not exhibit age-dependent behavioral decline. Based on the analysis of the mutants, we have been trying to discover the molecular link between the end of reproduction and behavioral decline. I want to discuss the possibility that behavioral aging can be genetically programmed to increase the fitness of a population by sacrificing aged individuals.

Presentation type: Invited speaker - regular talk

The cost of hosting a microbiome with age

Filipe Cabreiro

University of Cologne - CECAD

Prolongation of lifespan is one of the greatest achievements of modern medicine. As an unfortunate consequence, age-related diseases are thriving. A comprehensive understanding of the ageing process is therefore crucial for healthy ageing. While human host genetics are not straightforward to tinker with, diet and the microbiota are excellent untapped opportunities to improve disease states. Dysbiosis has been recently proposed as a hallmark of ageing through the development of a chronic low-grade inflammatory state, and may be one of the driving factors behind age-related diseases. Using model systems including germ-free and conventionally raised mitochondrial deficient progeria mouse models and C. elegans together with high-throughput discovery platforms, we investigate the role of gut microbiota in the development of age-related phenotypes to identify tissue cross-talk regulators modulated by the microbiota. The promising research endeavors in this area are the identification of early biomarkers and actionable mechanisms for improving healthy ageing.

Presentation type: Invited speaker - regular talk

Evolutionary and Ecology of Aging

Dario Riccardo Valenzano

Leibniz Institute on Aging

African killifishes have emerged over the past few years as a powerful model system to answer open questions in biology of aging, developmental and evolutionary biology. Killifish evolved in a range of environments, from rainforest to savannah water holes that desiccate seasonally. Killifish survive periodic desiccation by evolving an annual life cycle, characterized by a specialized embryonic adaptation (embryonic diapause), where embryos suspend development in the dry mud until the external conditions are safe for them to hatch. Annual killifish that evolved in dry environments often display short natural lifespan and a wide range of age-related changes, including neurodegeneration, inflammation, fibrosis, immune decline and dysbiosis. In my talk, I will share that i) studying killifish ecology and evolution has opened new perspectives to understanding that species' lifespan and aging evolve as a function of past demographic constraints, and that ii) an ecological perspective to killifish biology, which focuses on host-microbiome functional interactions,

opens new insights into biology of aging, as well as offers novel opportunities for anti-aging interventions.

Presentation type: Invited speaker - regular talk

Cell-molecular-biomechanics crosstalk ensuring age-progressive nervous system integrity

Georgia Rapti

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Proper tissue function relies on faithful assembly and resilient maintenance of tissue architecture. In the nervous system, failure to preserve integrity of circuit architecture upon environmental challenges and age progression is often associated with human neuropathology. However, mechanisms dedicated to preserving circuit integrity often remain elusive and challenging to study experimentally, due to circuit complexity, limited single-cell resolution accessibility, and the involved timescales. Neurons and glia both sculpt circuits and functional connectivity, but the mechanisms ensuring their long-term integrity in vivo, their crosstalk with extracellular matrix (ECM) and the environment, remain understudied. We use C. elegans as a powerful setting for single-cell-resolution investigations, and genetic tractability, with well-characterized anatomy, connectivity, and glial cells largely dispensable for neuronal birth and viability. We decipher mechanisms of circuit architecture by integrating synthetic biology, in vivo fluorescent and biomechanics imaging, and quantitative and physiological analysis. We isolated gene alterations resulting in defects in age-progressive integrity and characterized circuit architecture, alterations, and interactions. We identified age-progressive defects in glial cells and neuronal components, as well as associated defects of synapse healthspan and animal lifespan. By integrating manipulations of cells, genes and the environment we dissect an interplay of protein homeostasis, ECM, and tissue biomechanics that ensure circuit integrity. The underlying molecular factors include conserved heat-shock chaperone regulators of proteostasis, and enzymes catalyzing post-translational modifications. We identify defects of the ECM and tissue biomechanics that precede circuit integrity defects, and we can affect and safeguard the integrity of circuit architecture by manipulating ECM composition, cell junctions, or temperature and material properties of the

environment. Our studies dissect cellular and molecular mechanisms ensuring the integrity of circuit architecture across spatial and temporal scales and may provide insights for future studies of neurodegeneration and neuropathology.

Presentation type: Invited speaker - regular talk

Session IV | Morphogenesis and embryonic development

Cellular mechanisms of environmental control in thyroid morphogenesis

Asako Shindo , Mai Okada , Tadashi Watanabe , Taishi Hamaguchi
Osaka University

Embryonic morphogenesis is primarily driven by endogenous processes such as gene expression, but environmental factors can modulate certain organogenesis processes. We previously found that feeding-induced nutritional signals trigger thyroid follicle morphogenesis in Xenopus laevis tadpoles, indicating that the nutritional environment acts as a non-autonomous regulator of thyroid organogenesis. However, the cellular and molecular mechanisms underlying responses to these environmental cues remain unknown. Using Xenopus tadpoles, we have identified cell adhesion molecules as targets of nutritional signals during thyroid morphogenesis. Feeding reduced cell adhesion molecule expression in the thyroid region, whereas a lack of feeding increased their expression, impeding follicle enlargement. We also identified actomyosin cytoskeleton as a target of nutritional signals regulating thyroid organ shape. Thyroids of fed tadpoles had smooth surfaces, while actomyosin inhibition caused rough surfaces with protruding structures. Similarly, unfed tadpoles' thyroids displayed a distorted surface with protrusions. Furthermore, live imaging of thyroid tissues from fed tadpoles revealed distinct cell movements driving thyroid follicle expansion independently of cell proliferation. These findings highlight that changes in cell adhesion and cytoskeletal dynamics play a crucial role in regulating cellular and tissue-level morphogenesis in response to nutritional status. We are further analyzing whole-body 3D imaging to identify organs beyond the thyroid that are sensitive to nutritional status. I will

also discuss unexplored morphogenetic processes emerging from interactions between environment and organ development.

Presentation type: Invited speaker - regular talk

Multiscale synthesis of coupled dynamic gene expression during neural development

Nancy Papalopulu

University of Manchester

In recent years, our understanding of how cells transition from proliferation to differentiation has been transformed by the application of single cell quantitative approaches and live imaging of protein expression with knocked-in reporters. Such approaches have led to by discovery of short-time scale protein expression oscillations which are exemplified by the expression of the Notch target HES/Her transcription factors in neural progenitors. In my talk, I will show how single cell oscillations of HES5 undergo multiscale synthesis when cells interact in a tissue environment and how the dynamic emergent pattern may control the rate and spreading of differentiation events in space.
A computational model where Notch signalling is extended to non-neighbouring progenitor cells combined with a regular perturbation of Notch signalling by the emerging differentiating cells, is sufficient to recapitulate the complex emergent tissue-level of HES5 dynamics. I will provide experimental evidence that begins to validate these computational findings. In particular, I will show the existence of cellular protrusions all along the apicobasal axis of neural progenitors, that extend cell contacts beyond immediate neighbours, and may deliver Notch signalling at a distance to modulate the tissue level pattern of HES5.

Presentation type: Invited speaker - regular talk

Integrative approaches to tissue morphogenesis

Elias H Barriga

Cluster of Excellence Physics of Life (PoL), TU Dresden, Dresden, Germany

Synchronisation of morphogenetic events is essential to secure robust morphogenesis. In my talk I will discuss our integrative approaches about the mechanisms underlying the spatio-temporal coordination of collective behaviours during tissue morphogenesis. Briefly, I will provide examples of our

work in which we explore how biophysical proportion emerge from living tissues and integrate with genetic frameworks to coordinate transitions of cells and tissue states at the right time and the right place.

Presentation type: Invited speaker - regular talk

The role of multiple critical points in embryonic tissue phase transitions

Laura Rustarazo-Calvo (1,2) , Cristina Pallares-Cartes (1) , Adrián Aguirre Tamaral (3) , Elisa Floris (3) , Maximilian Hingerl (1,2) , Bernat Corominas Murtra (3) , Nicoletta Petridou (1)

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Material phase transitions, where tissues switch between fluid-like and solid-like states, are central to morphogenesis. These transitions occur at the critical points of multiple cell control parameters such as density, shape, adhesion, and contact dynamics. However, whether these parameters and the transitions across their corresponding critical points are interdependent and serve redundant or distinct functions remains unclear. Here, we show that embryonic tissues regulate their material state by coupling transitions across multiple critical points, and that uncoupling them alters the tissue morphogenetic outcome. Using phase transition theoretical frameworks, we identify that the critical points of three control parameters — cell density, adhesion, and connectivity — are crossed concurrently during zebrafish morphogenesis. By employing genetics, optogenetics, pharmacological perturbations, and biophysical measurements, we independently modulate each control parameter and disentangle their individual contributions to tissue rheology, revealing that cell adhesion is the sole determinant of the tissue material state. Remarkably, by fine-tuning different control parameters relative to their critical points, we identify combinations that drive tissues to acquire distinct morphological and molecular features, including mesenchymal, epithelial, and luminal structures. These findings uncover a novel mechanism in which the coordinated regulation of diverse cell control parameters with respect to their critical points governs the morphogenetic landscape of embryonic tissues.

Presentation type: Invited speaker - abstract talk

Session V | Tissue and organ morphogenesis and homeostasis

Integrated Omics Analysis of Hair Follicle Development

Ritsuko Morita
Osaka University

Skin and hair follicles are pioneering model organs that have driven the development of stem cell biology. However, when we started our research, the developmental processes of skin and hair follicles were not fully analyzed. By combining single-cell resolution live imaging with single-cell transcriptomics, we have captured changes in cell lineage and gene expression during the development of mouse hair follicle epithelium, revealing new insights into hair follicle developmental patterns and the developmental origin and formation process of hair follicle stem cells.
As hair follicle development involves epithelial-mesenchymal interactions, we are currently in the process of advancing an integrated analysis that combines single-cell transcriptomics, live imaging, and spatial analysis. This work aims to uncover the spatio-temporal epithelial-mesenchymal interactions that contribute to hair follicle stem cell formation. In this talk, I will present our findings to date and discuss the future directions of this research.

Presentation type: Invited speaker - regular talk

Brillouin microsscopy reveals highly dynamic mechanical transitions during Drosophila gastrulation

Robert Prevedel
EMBL

During animal development, the acquisition of three-dimensional morphology is a direct consequence of the dynamic interaction between cellular forces and the mechanical properties of cells and their environment. While the generation and transmission of cellular forces has been widely explored, less is known about the dynamic changes in cell mechanical properties during morphogenesis. Here, we characterise and spatially map in three dimensions the dynamics of cell mechanical properties during Drosophila gastrulation utilising line-scan Brillouin microscopy. We find that cells in the embryo undergo rapid and spatially varying changes in their mechanical properties and that these differ in cell populations with different fates and behaviours. We

identify microtubules as potential effectors of cell mechanics in this system, and corroborate our experimental findings with a physical model that underscores the role of localised and dynamic changes in mechanical properties to facilitate tissue folding. Our work provides the first spatio-temporal description of the evolving mechanical properties of cell populations during morphogenesis, and highlights the potential of Brillouin microscopy in studying the dynamic changes in cell shape behaviours and cell mechanical properties simultaneously in different cell populations in an intact organism.

Presentation type: Invited speaker - regular talk

Harenessing Stem Cell Death for Tissue Regeneration

Yaron Fuchs

Augmanity, Rehovot, Israel

What if cell death wasn't just an endpoint, but a catalyst for regeneration? This talk will explore the remarkable role of programmed cell death in driving tissue repair and renewal. From "super-competitive" stem cells that eliminate less-fit neighbors to apoptotic signals that stimulate proliferation and new hair follicle formation,I will discuss how cell death orchestrates regeneration at the molecular and cellular levels.

Presentation type: Invited speaker - regular talk

Mechanochemical coupling during intestinal crypt morphogenesis

Laura Capolupo [2], Clara Baader [1, 3], Paul Robin [4], Silvia Barbiero [1, 3], Qiutan Yang [5], Koen Oost [1], Jennifer Groeli [1], Edouard Hannezo [4], Prisca Liberali [1, 2, 3]

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Intestinal development represents a prototypical example of spatio-temporally coordinated morphogenetic events that lead to the emergence of cell types and the stereotypical crypt-villus axis. Thanks to organoid technology we can model this process and investigate the biochemical and mechanical modulations that drive it. Crypt morphogenesis is started by actomyosin-driven apical

constriction and accelerated by osmotically driven epithelial volume changes. However, what remains to be understood is how these mechanical and osmotic cues are coordinated and then translated into morphogenic programs and how well this mechano-osmotic changes are recapitulated in vivo during development to ensure a timely and synchronized crypt formation. Here we combine scRNAseq, quantitative morphometric analysis, biophysical modelling, and organoid technology to identify the molecular mechanism responsible for the feedback between mechano-osmotic inputs and intestinal morphogenesis both in vitro and in vivo. We show that crypt morphogenesis during development entails morphological changes similar to those observed in organoids and that the increase in curvature, cell compaction and density together with changes in osmolarity are tightly correlated and drive apical constriction and crypts formation. Importantly, we identified the calcium-dependent cytosolic phospholipase A2 (cPLA2) as the factor responsible for mechano-osmotic driven crypt formation. Indeed, cPLA2 is specifically expressed in Paneth cells and Stem cells where it gets activated upon tissue and cell shape changes and osmotic variations, to produce arachidonic acid. Finally, we show that cPLA2 activation induces crypt morphogenesis and its robustness, by promoting myosin relocalization and actomyosin-driven apical constriction. Together, our data uncover a critical mechanochemical feedback mechanism that senses and integrates morphological and mechano-osmotic changes to drive crypt morphogenesis and lead to an irreversible and robust configuration.

Presentation type: Invited speaker - abstract talk

Session VI | Vascular endothelial cells development and regulation in health and disease

A novel role of endothelial cells in alveolar morphogenesis

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Endothelial cells (ECs) construct blood vessel networks to deliver blood, providing oxygen and nutrients to tissues throughout the body. Beyond this fundamental role, ECs also produce angiocrine factors that regulate organ development, maintenance, and regeneration. In this study, we demonstrate that ECs contribute to organ morphogenesis not only by secreting angiocrine factors but also through their intrinsic cellular functions.
Alveologenesis is a spatially coordinated morphogenetic event that generates alveoli, creating a large surface area for gas exchange in lungs. In this process, alveolar myofibroblasts surround terminal sacs formed by alveolar epithelial cells and ECs, then contracting to generate alveoli through mechanical signaling driven by actomyosin activation and YAP nuclear localization.
Rap1, a small GTPase belonging to Ras superfamily, is known to stimulate integrin binding to the extracellular matrix, thereby promoting cell adhesion. Recently, we found that EC-specific Rap1-deficient mice exhibited defective alveolar formation due to the decreased mechanical signaling in myofibroblasts. Mechanistically, we discovered that ECs construct basement membranes (BMs) which act as a scaffold for myofibroblasts to induce alveolar formation through activation of mechanical signaling. We further showed that endothelial Rap1 activates integrin β1, which recruits collagen type IV (Col-4) to generate BMs. Consistently, EC-specific Itgb1-deficient mice displayed hypo-alveolarization, impaired mechanical signaling in myofibroblasts, and disorganized BMs, similar to EC-specific Rap1-deficient mice.
Collectively, these results demonstrate that alveolar ECs promote integrin β1-mediated Col-4 recruitment in a Rap1-dependent manner, thereby constructing BMs that serve as a scaffold for myofibroblasts to induce mechanical signal-driven alveologenesis. Thus, our study unveils a novel mechanism of EC-mediated organ development driven by their intrinsic cellular functions.

Presentation type: Invited speaker - regular talk

Vascular heterogeneity and specialization in development and disease

Michael Potente
Angiogenesis & Metabolism Laboratory
Berlin Institute of Health at Charite & Max Delbrück Center for Molecular Medicine

Blood and lymphatic vessels pervade almost all body tissues and have numerous essential roles in physiology and disease. The inner lining of these networks is formed by a single layer of endothelial cells, which is specialized according to the needs of the tissue that it supplies. Whereas the general mechanisms of blood and lymphatic vessel development are being defined with increasing molecular precision, studies of the processes of endothelial specialization remain mostly descriptive. Recent insights from genetic animal models illuminate how endothelial cells interact with each other and with their tissue environment, providing paradigms for vessel type- and organ-specific endothelial differentiation. Delineating these governing principles will be crucial for understanding how tissues develop and maintain, and how their function becomes abnormal in disease.

Presentation type: Invited speaker - regular talk

Vascularising the heart - one cell at a time

Holger Gerhardt
Max-Delbrück-Center for Molecular Medicine, Berlin, Germany

Presentation type: Invited speaker - regular talk

A multimodal analysis of epithelial cell lineage in the human embryonic lung

John Russell [1], Richard Butler [1], Anthony Sinadinos [3], Uta Griesenbach [3], Eric Alton [3], Emma Rawlins [1, 2]

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The conducting airways are established by branching morphogenesis during the embryonic stages of development. Airway growth is driven by a population of multipotent SOX9+ progenitors that produce all cells of the epithelium, the

composition of which changes significantly from proximal to distal. How this pattern is established in humans has not been adequately described due to a lack of experimental tools. Moreover, interspecies differences in both cell types and organ morphology limits the utility of other model organisms. Here, we present a multimodal workflow to characterise cell lineage hierarchies in developing branching organs. We have developed a graphical user interface for integrating multiple wholemount datasets to characterise cell specific markers as a function of airway generation, creating a 3D spatio-temporal map of cell emergence in the embryonic human lung. Complemented by single cell transcriptomic datasets, we used this tool to describe transient cell states in the distal airways preceding lineage commitment. We further optimised the culture of intact human embryonic lobes that branch and maintain epithelial patterning over several days in culture. Utilising lentiviruses pseudotyped to target airway epithelia, we performed unbiased lineage tracing at clonal resolution. This revealed that clonal composition changes as a function of airway generation and uncovered lineage relationships between ASCL1+ pulmonary neuroendocrine cells, P63+ basal cells and SCGB3A2+ secretory progenitors. Finally, we have demonstrated by small molecule inhibition that the NOTCH pathway has a duel function for establishing cell fate in the distal airways and maintaining cell state in the proximal airways. Together these tools provide a basis for studying cell lineages in intact branching organs which have uncovered lineage relationships previously undescribed in the embryonic human lung.

Presentation type: Invited speaker - abstract talk

Session VII | Computational genomics - tools and technologies

Deep learning-based approaches to elucidate unknown functional regions of the genome

Ryuichiro Nakato
Institute for Quantitative Biosciences, University of Tokyo, Japan

The field of epigenomics is advancing rapidly, driven by international database projects such as the International Human Epigenome Consortium (IHEC) and ENCODE. These resources enable the development of deep learning (DL)-based approaches to extract new biological insights from large datasets. Here, I present our two computational approaches using DL for epigenome analysis. First, for the functional annotation of uncharacterized genomic regions, we use transformer-based models such as Enformer, which predict the read distribution of transcriptome and epigenome. These models can provide contribution scores along the input genomic sequence, allowing assessment of their functional potential in terms of gene expression and protein-binding intensity. Here I will present our trial targeting RNA Pol II elongation. Second, I present our new BERT-based model, ChromBERT [1], which converts chromatin state annotations (e.g., promoters and enhancers) into alphabetical sequences and identifies distinct patterns in "chromatin state sequences." ChromBERT also employs Dynamic Time Warping (DTW) for motif clustering to improve interpretability. These tools demonstrate the potential of novel approaches to epigenomic analysis and offer new perspectives for functional genomic studies.
[1] Lee S, Lin C, Chen CY, *Nakato R. ChromBERT: Uncovering Chromatin State Motifs in the Human Genome Using a BERT-based Approach, bioRxiv, July 26, 2024.

Presentation type: Invited speaker - regular talk

Dicing and scrambling the genome to probe and model gene regulation

Bas van Steensel
Netherlands Cancer Institute and Oncode Institute

One of the major challenges in genomics is to accurately predict genome-wide gene expression from the sequences of regulatory elements. I will discuss two strategies that we are taking to tackle this challenge.

First, we have developed PARM, a cell-type specific deep learning model that reliably predicts promoter activity throughout the genome from DNA sequence alone. This model is trained on activity measurements of millions of human DNA fragments. PARM is a powerful tool to uncover the mechanisms that govern regulation of mammalian promoters.
Second, we have developed an approach to relocate regulatory elements such as enhancers and promoters in the mouse genome to hundreds of alternative positions and measure the impact on gene activity. We thus obtain high-resolution functional maps of a genomic locus that help to understand the logic of the positioning of regulatory elements relative to their target gene.

Presentation type: Invited speaker - regular talk

Why do chromosomes look like chromosomes (not like a ball of yarn)?

Frank Uhlmann
The Francis Crick Institute, London, UK

Chromosomes in different organisms adopt various dimensions, the determinants for which are poorly understood. E.g. the recently diverged Chinese and Indian muntjacs contain genomes of similar size distributed amongst 46 or only 6 chromosomes, respectively. The 6 Indian muntjac chromosomes are much thicker than the Chinese muntjac chromosomes to accommodate the required length of DNA. Here, we investigate how chromosome width is defined.
We begin by comparing budding yeast and fission yeast, which harbour similarly sized genomes distributed amongst 16 or 3 chromosomes. Genomic approaches reveal a species-specific chromosome width determinant, which we find correlates with the spacing intervals between binding sites of the chromosomal condensin complex. Unexpectedly, even within each species, longer chromosome arms are always thicker. We use this information to explore molecular models for how mitotic chromosomes gain their shape.

Presentation type: Invited speaker - regular talk

Origins of chromosomal instability in human cells unveiled by coupled
AI, imaging and genomics

Jan Korbel

EMBL

Chromosomal instability results in widespread structural and numerical chromosomal abnormalities (CAs) during cancer evolution. While CAs have been linked to mitotic errors resulting in the emergence of nuclear atypias, the underlying processes and basal rates of spontaneous CA formation in human cells remain under-explored. Here we introduce machine learning-assisted genomics-and-imaging convergence (MAGIC), an autonomously operated platform that integrates automated live-cell imaging of micronucleated cells, machine learning in real-time, and single-cell genomics to investigate de novo CA formation at scale. Applying MAGIC to near-diploid, non-transformed cell lines, we track CA events over successive cell cycles, highlighting the common role of dicentric chromosomes as an initiating event. We determine the baseline CA rate, which demonstrate approximately doubles in TP53-deficient cells, and show that chromosome losses arise more rapidly than gains. Furthermore, we show that the targeted induction of DNA double-strand breaks along chromosomes triggers distinct CA processes, revealing stable isochromosomes, amplification and coordinated segregation of isoacentric segments in multiples of two, and complex CA outcomes, depending on the break location. Our data contrast de novo CA spectra from somatic mutational landscapes after evolutionary selection occurred. The large-scale experimentation enabled by MAGIC provides insights into de novo CA formation, paving the way to unravel fundamental determinants of chromosome instability.

Presentation type: Invited speaker - regular talk

Session VIII | Cytoskeletal functions

Building up cell motility in synthetic cells

HIdeaki Matsubayashi
Frontier Research Institute for Interdisciplinary Sciences, Tohoku University

Cell migration is a dynamic process emerging from the coordinated dynamics of the actin cytoskeleton. Although key molecules involved in force generation at the leading edge of cells have been identified, the minimal set of factors required to drive unidirectional motion in cell-sized lipid compartments remains elusive. Aiming to reconstitute cell motility, we developed a novel molecular tool to induce actin polymerization within living cells and then applied this approach to giant unilamellar vesicles (GUVs). Leveraging spatiotemporal control of actin assembly, we found that directed reorganization of actin networks led to outward membrane protrusions and propelled GUVs at speeds up to 0.43 μm/min—comparable to adherent mammalian cells. Notably, our findings reveal a synergistic interplay between branched and linear actin structures in promoting membrane extensions, underscoring their cooperative nature. This strategy provides a powerful platform for dissecting the fundamental mechanics of cell migration, engineering synthetic cells with active morphodynamics, and advancing bioengineering applications.

Presentation type: Invited speaker - regular talk

A molecular atlas of the cell motility machinery

Florian Schur
Institute of Science and Technology Austria

Cell motility depends on the protrusive force generated by the actin cytoskeleton at the leading edge of cells. Actin forms higher-order networks with versatile physical properties which are attributed to the underlying actin filament biochemistry and actin filament network geometry. We currently still lack an understanding of the full complexity of involved actin filament populations that define how protrusive force is generated and how different migratory properties arise.
To overcome these limitations, we establish novel workflows integrating optimized specimen preparation, cryo-electron tomography data collection and image processing to perform large-scale molecular imaging and a molecular census of the cellular leading edge. This data enables us to quantify the actin network within the entire cell leading edge and to provide a ground-truth

understanding of how the geometrical complexity of the actin cytoskeleton steers directional cell migration.

Presentation type: Invited speaker - regular talk

Collective cell motion and symmetry breaking in multicellular systems

Pierre-François Lenne

IBDM and Turing Center for Living Systems, Aix Marseille Univ, CNRS

During early development, multicellular animals self-organize to establish body axes, such as the head-to-tail axis. Several signaling pathways are known to control body axis formation. However, we show that tissue mechanics also plays an important role during this process.
Focusing on spherical aggregates of mouse embryonic stem cells, we show how these organoids break rotational symmetry to form an axial organization with domains of different expression profiles, e.g. the transcription factor T/Bra and the adhesion molecule E-cadherin
Quantitative microscopy and modeling approaches reveal large-scale tissue flows with a recirculation component contributing to symmetry breaking, driven by differences in tissue surface tension, akin to Marangoni flows.
Our work highlights that body axis formation is not only driven by biochemical processes, but that it can also be amplified by tissue flows. We expect that this type of amplification may operate in many other organoids and in vivo systems.

Presentation type: Invited speaker - regular talk

The missing link of surface mechanics: Membrane-to-cortex attachment protein caging triggers symmetry breaking in cells

Srishti Dar

Cell Biology and Biophysics Unit, European Molecular Biology Laboratory, Heidelberg, Germany

Animal cell shape changes are primarily driven by its surface, a composite interface comprising a thin cortical actin network physically tethered to the plasma membrane by specialized membrane-to-cortex attachment (MCA) proteins. Previous studies have focused on how gradients in myosin motors or actin network architecture drive cell deformations, overlooking any mechanical

contribution of the membrane per se. Here, we identify membrane viscosity and the length of MCA proteins as additional features that synergistically regulate cortical dynamics, leading to cell polarity in cells. By combining in silico, in vitro and in cellula approaches we show that contractility in actomyosin networks scales linearly with membrane viscosity when MCA proteins are shorter than the distance between the membrane and the cortical actin network (MC-distance). However, contractility changes by several orders of magnitude longer linker proteins due to their caging within the cortex, ultimately blocking actin network remodelling by myosin motors. On the basis of our findings, we propose that local changes in lipid composition, in combination with the choice of MCA protein, allow cells to locally regulate cortical mechanics, leading to cellular morphogenesis.

Presentation type: Invited speaker - abstract talk

Session IX | Ribosome and protein biogenesis - from structures to mechanisms

Ribosome biogenesis in vitro

Wataru Aoki

Osaka University

Ribosome biogenesis is pivotal in the self-replication of life. In Escherichia coli, three ribosomal RNAs and 54 ribosomal proteins are synthesized and subjected to cooperative hierarchical assembly facilitated by numerous accessory factors. Realizing ribosome biogenesis in vitro is an essential goal to understand the self-replication of life and creating artificial cells; however, this has not been realized because of its complexity. We have reported the first successful ribosome biogenesis in vitro. Specifically, we developed a highly specific and sensitive reporter assay for the detection of nascent ribosomes. The reporter assay allowed for combinatorial and iterative exploration of reaction conditions for ribosome biogenesis, leading to the simultaneous, autonomous synthesis of both small and large subunits of ribosomes in vitro through transcription, translation, processing, and assembly in a single reaction space. Our

achievement represents a critical step toward revealing the fundamental principles underlying the self-replication of life and creating artificial cells.

Presentation type: Invited speaker - regular talk

Co-translational protein maturation at the ribosomal tunnel exit

Irmgard Sinning
Heidelberg University Biochemistry Center

Already during translation nascent chains are subject to enzymatic modification, folding and targeting by large set of ribosome associated factors (RAFs). These factors need to be coordinated at the crowded environment of the tunnel exit to ensure their timely recruitment, correct function, and eventual displacement. In eukaryotes, this pool of factors includes Methionine Aminopeptidases (MAPs), N-terminal Acetyltransferases (NATs), N-Myristoyltransferases (NMTs), the ribosome associated complex (RAC), the nascent polypeptide associated complex (NAC), the signal recognition particle (SRP), the translocon (SEC61) and others. Most nascent chains undergo several successive maturation steps before they are released from the ribosome. Here, we utilize single particle cryo-EM and biochemical assys to study the interaction of the enzymes with the ribosomal PTE. Our structures reveal that RAFs influence each other at the tunnel exit and can form large multi-protein assemblies in preparation for an emerging nascent chain. These assemblies are inherently dynamic and can assume a range of conformations at the ribosomal tunnel exit.

Presentation type: Invited speaker - regular talk

Studying ribosome-targeting antibiotics in context to infer their mechanisms of action

Axel Innis
ARNA Laboratory, INSERM U1212 – CNRS UMR5320, Univ. Bordeaux (France)

Antibiotics are essential for modern medicine, but drug-resistant pathogens are increasingly threatening their effectiveness. Although current ribosome-targeting antibiotics could be improved through structure-guided design, many are poor candidates for such a knowledge-based approach, as their mechanisms of action are incompletely understood. This is particularly true of drugs that do not block the synthesis of all proteins with equal efficacy, but rather depend on

a particular translational context, such as the amino acid sequence of the nascent peptide or the nature of the tRNAs present in the ribosome. Therefore, establishing the context dependence of ribosome-targeting antibiotics ensures that functionally relevant complexes are used to determine their mechanisms of action. Here, I will present our efforts to assess the context dependence of antibiotics, old and new, as a prelude to determining their detailed mechanisms of action.

Presentation type: Invited speaker - regular talk

Visualizing translation regulation in a minimal bacterium

Joe Dobbs

EMBL

Recent advances in cryogenic electron tomography (cryo-ET) are now allowing researchers to structurally characterize macromolecular complexes in situ at increasingly high resolution, investigate interaction networks and protein communities, and derive functional insight from dynamic and heterogenous assemblies unsuitable for traditional structural methods. Here, we present our investigation into the molecular sociology of gene expression, focusing on the interaction of transcriptional and translational machineries in the minimal prokaryote Mycoplasma pneumoniae. M. pneumoniae has been used recently by our group in studies of the ribosome, providing the first detailed snapshots of translation elongation inside the cell, and our new analysis is expanded to encompass the entire cycle of prokaryotic translation, visualizing and quantifying translation initiation, elongation, and recycling complexes. Our findings implicate noncanonical translation initiation. Traditionally, the Shine-Dalgarno (SD) sequence, a common motif in prokaryotic mRNAs, has been thought to be the primary driver of mRNA delivery to the bacterial ribosome, but bioinformatics analyses have demonstrated that across all prokaryotic genomes only a minority of open reading frames (ORFs) are preceded by this sequence element. M. pneumoniae, in particular, has a low percentage (8.1%) of SD-sequence ORFs, and most of these occur in the coding region: we show that the bacterium has a high frequency of transcription-translation coupling during translation initiation (in addition to elongation), and that the interaction between RNA polymerase and the ribosome occurs differently in cells of different sizes. Furthermore, we suggest, with antibiotic perturbations and structural evidence, that some of the mechanisms involved are substantially different from those typically understood to be occurring in prokaryotes.

Presentation type: Invited speaker - abstract talk

Session X | Plant biology

From chaos to order: cell fate specification and self-organization in pluripotent callus during organ regeneration in plants

Momoko Ikeuchi , Yuki Doll

Nara Institute of Science and Technology

Plants have an outstanding regenerative capacity to re-construct brand-new individuals from tissue explants. In a tissue culture-based de novo shoot regeneration, shoot apical meristems (SAM) harboring persistent stem cells spontaneously arise from pluripotent cell mass called callus. It remained unknown how the developmental fates of callus cells are specified and self-organize to establish SAM de novo. We recently characterized the dynamics of callus cell population using highly-sensitive single cell transcriptome analyses combined with imaging approaches (Ogura et al., 2023 Sci. Adv.). We proposed that a mutually repressive regulatory module between two homeobox transcription factors, WUSCHEL (WUS) and WUSCHEL-RELATED HOMEOBOX 13 (WOX13), regulate the binary fate specification in callus cell population. WUS is well characterized as a central regulator of SAM establishment, whereas WOX13 has been recently identified to play pivotal roles in tissue repair and grafting by our studies (Ikeuchi et al 2022 Plant Physiol.; Tanaka et al. 2023 PCP). Recently, we also found that cellular fates are strongly influenced by the surrounding cells. In this talk, we will discuss potential mechanisms underlying pattern formation which enable de novo organogenesis in plants.

Presentation type: Invited speaker - regular talk

Role of ANAC transcription factors in ribosomal stress response in *Arabidopsis*

Christian Wenzl , Jan U Lohmann

Center for Organismal Studies (COS)

Ribosomal RNAs, as well as proteins, are assembled into functional ribosomes in the nucleolus in a process called ribogenesis. Disruption of this process leads to ribosomal stress, which induces specific cellular responses aimed at maintaining cell homeostasis. In animal cells, the ribosomal stress response is mediated by activation of the tumor suppressor *p53*, leading to cell cycle arrest or programmed cell death.

In plants, mutants with impaired ribogenesis often share a common phenotype, which indicates the existence of a stress response pathway connecting ribogenesis and plant growth. In the absence of a *p53* homolog, it has been shown only recently that ANAC082, a member of the plant-specific NAC transcription factor family, is a key regulator of the ribosomal stress response in *Arabidopsis*.
We have identified a novel hypomorphic allele in *rrp5*, an essential gene involved in ribosomal RNA processing. While $rrp5^{\Delta 10}$ mutants exhibit an overall wild-type appearance, we observed specific cell morphological changes in the epidermal cells of the shoot apical meristem. Transcriptome and reporter construct analysis revealed a strong induction of members of the stress-associated DUF295 gene family, sharing a conserved ANAC binding site in their promoter region. We could show that this binding site is sufficient to induce DUF295 expression in $rrp5^{\Delta 10}$ as well as in $rrp7^{1}$ mutants. Not unexpectedly mutations in *anac082* can suppress ribosomal stress induced phenotypes in both mutants. However, we observed that mutations in *anac044*, *anac085*, and *anac103* can also suppress these phenotypes, indicating that cell- and tissue-specific ribosomal stress responses in plants are mediated by a complex transcriptional machinery.

Presentation type: Invited speaker - regular talk

Short and long distance signalling in plants during drought stress

Rüdiger Hell

Centre for Organismal Studies, Heidelberg University, Germany

Presentation type: Invited speaker - regular talk

Stem cell factor induced regeneration in plants

Jana Wittmer

Cell and Developmental Biology, cluster Plant Developmental Biology, Wageningen University & Research, Droevendaalsesteeg 1, 6708 PB, Wageningen, The Netherlands

In the plant kingdom pluripotent stem cells are maintained in the stem cell niches throughout a plants life cycle. Under suitable in vitro conditions involving the application of phytohormones, pluripotency can be equally induced from differentiated plant cells. However, regenerative recalcitrance to respond to tissue culture manipulation presents a bottleneck that lies in the

species, cultivars and explant origin. Here we took an approach similar to the induced pluripotent stem cell concept in animals and overexpress a set of root stem cell specific factors in Arabidopsis thaliana, to induce and study regeneration in the absence of hormones. We find that regeneration induced by these factors goes via the somatic embryogenesis program. We provide evidence that this setup can be translated to crops using the same set of factors by regenerating lettuce on hormone-free media

Presentation type: Invited speaker - abstract talk

Flash talks

Integrative Gene Clustering Analysis of Coessentiality and Expression Data Using Graph Neural Network

Gina Oba , Ryuichiro Nakato
Institute for Quantitative Biosciences, University of Tokyo

Annotating gene function is key to understanding complex cellular phenotypes. Network analysis has become a powerful tool for predicting the functions of previously uncharacterized genes. The approach, known as guilt by association, assumes that genes closely connected within a network tend to share similar functions. Co-expression networks, derived from gene expression patterns across multiple cell types, are often used to identify functional gene groups. Recent research shows that Coessentiality networks, constructed from correlations in fitness effects of single-gene perturbations across diverse cancer cell types, have been shown to group genes into protein complexes more effectively than co-expression networks. However, these networks do not represent cell-type-specific information, limiting their ability to identify genes with functions that depend on cellular context.
To address this, we tested an integrative analysis combining coessentiality and gene expression data across cancer cell line data. By integrating cell-type-specific contexts, our approach aims to uncover complex, cell-type-dependent gene regulatory mechanisms, particularly within protein complexes and context dependent usage of paralog genes. Using data from the Cancer Dependency Map (DepMap 23Q2), including essentiality scores for 17,656 genes across 964 cancer cell lines, we constructed two gene networks: one with

coessentiality as edges and expression patterns as node features, and another with co-expression as edges and essentiality patterns as node features.
We applied a Graph Neural Network (GNN) model for unsupervised node embedding and clustering to identify biologically meaningful gene modules. This method has the potential to provide insights into context-dependent functions, such as the cell-type-specific utilization of paralogs or functional differentiation across cell cycle stages, and to reveal how gene functions are modulated by cellular context. This framework advances the study of protein complexes across diverse biological contexts.

Immune Regulation with mRNA: Inducing Antigen-Specific Tregs for Therapeutic Applications.

Shota Imai [1, 2], Tomoyoshi Yamano [1, 3], Rikinari Hanayama [1, 3]
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[2] Nano Life Science, Graduate School of Frontier Science Initiative, Kanazawa University
[3] WPI Nano Life Science Institute (NanoLSI), Kanazawa University

We aimed to develop a safer approach to induce antigen-specific regulatory T cells (Tregs) in vivo for treating autoimmune diseases and allergies. Existing therapies, like immunosuppressive drugs, lack specificity and cause serious side effects. Tregs are key in maintaining immune tolerance and preventing autoimmune disease, but inducing antigen-specific Tregs in vivo remains challenging. We developed mRNA-Treg, encoding immune regulatory molecules along with antigens, to enhance Treg induction. Using the MOG antigen, a mouse model of multiple sclerosis, mRNA-Treg induced MOG-specific Tregs more efficiently than conventional mRNA and led to their accumulation in both the spleen and spinal cord. These Tregs expressed markers like CD25 and CTLA4 and reduced inflammatory cytokine release (IFN-γ, IL-2), showing improved safety over traditional methods. Moreover, mRNA-Treg prevented severe disease progression in an experimental autoimmune encephalomyelitis (EAE) model, whereas conventional MOG-mRNA did not. The successful induction of Tregs using another autoantigen and foreign antigens associated with autoimmune diseases and allergies indicates that mRNA-Treg has potential for use in both autoimmune and foreign antigen-associated diseases. Future studies will focus on assessing broader applicability and safety in human clinical trials.

Conversion into hepatocyte from intestinal regenerative stem cells

Fumiya Uefune
Institute of Science Tokyo

The transplantation of hepatocytes is the only curative treatment for acute liver failure, but the problem of donor shortage remains. Regenerative medicine using hepatocytes derived from ES- and iPS-cells has been intensively investigated, however there are still some issues before practical implementation.
Intestinal epithelium is highly regenerative cells. LGR5-expressing intestinal stem cells at the bottom of crypt play the essential role in maintaining homeostasis (Barker et al., 2007), while distinct stem cells are involved in regeneration of intestinal damage. We previously reported that Sca1/Ly6a expressing fetal-like stem cells emerge through activation of YAP/TAZ by extracellular matrix remodeling during wound repair, and this fetal-like reprogramming is reproduced by culturing epithelial cells in collagen type I-based gel instead of Matrigel in vitro (Yui et al., 2018, Kobayashi et al., 2022). These cells are called regenerative stem cells and valuable for the elucidation of the mechanism of intestinal regeneration associated with inflammation. Regenerative stem cells have the characteristics of decreasing the expression of mature intestinal epithelium-specific genes simultaneously with acquiring the fetal-like gene expression. Thus, we expect the potential of regenerative stem cells to convert into hepatocytes, which derived from common origins and have similar characteristics. Here we demonstrate the results of our investigation to convert collagen organoids established from mouse intestinal epithelial cells and human duodenum into hepatocytes. At first, we compared the gene expression of organoids cultured in collagen I with Matrigel. Collagen sphere increased the liver specific genes than Matrigel organoids as well as fetal-like signatures. Then we spread collagen sphere to collagen-coated plates with hepatocyte induction medium. The seeded cells survived for at least two weeks, and the expression of Albumin was upregulated. This study presents the potential of intestinal stem cells as new resources of liver transplantation and will lead to the elucidation of the factors determining tissue specificities.

A non-canonical Hedgehog-like pathway drives C. elegans embryonic brain assembly

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Effective tissue functionality relies on their complex architecture that emerges through cellular and molecular interactions of their components. Neural circuits consist of numerous, diversified neuronal and glial cell types, that interact to assemble complex functional architecture. Mechanisms of early circuit assembly are often challenging to study in vivo due to in-accessibility of embryonic stages for imaging and genetic manipulations. Moreover, glial cells, the non-neuronal half of circuits, remain understudied. C. elegans offers a powerful setting to study molecular underpinnings across scales of genes-cells-tissues. We study early circuit assembly events, using advanced genetics, real-time quantitative imaging, and behavioral studies.
We demonstrated that the C. elegans brain neuropil, consisting of 183 axons and 4 astroglial cells, forms in a hierarchical manner. We identified specific pioneer neurons and glia which drive assembly, guiding follower components in stereotypical spatiotemporal patterns. We characterized pioneer-cell-derived molecular cues driving assembly and revealed that an array of molecular synergies ensures fidelity of circuit assembly. To identify hidden factors underlying these synergies driving precise assembly, we developed modifier genetic screens of mutagenesis in sensitized backgrounds of mutants with mild defects, and combine them with targeted gene manipulation and in vivo quantitative imaging. In this way we identified genes of the non-canonical Hedgehog (Hh)-like pathway involved in the development of the C. elegans brain-like circuit. We show how different parts of this pathway play roles in embryonic pathfinding of specific neuronal types, and astroglial cell development. We also dissect the spatiotemporal roles and interactions of contributing pathway components. Altogether, our results demonstrate that the non-canonical Hh-like pathway drives molecular interactions of embryonic epidermis, astroglia and neurons, essential for fidelity of brain-like circuit development. Interestingly, this Hedgehog-like pathway is highly expanded in certain parts while lacking certain factors and we may provide insights on its evolutionary ancestral functions beyond C. elegans.

Poster Session

foundingGIDE: Founding a Global Image Data Ecosystem

Aastha Mathur
Euro-BioImaging ERIC

foundingGIDE is a Horizon Europe-funded project coordinated by Euro-BioImaging ERIC that will build strong foundations of a Global Image Data Ecosystem (GIDE) for image data exchange based on global coordination of technical developments among infrastructures and communities.
While being one of the fastest growing types of research data, imaging data lacks established standards, making compatibility among regional and national data resources a challenge. Although standardisation efforts are being made by various community initiatives, the necessary coordination of development activities is missing leading to a fragmented landscape with lack of awareness about existing solutions among researchers, and limited understanding of community needs by funders and policy-makers.
To address these challenges, the foundingGIDE project brings European research infrastructure and image data resource owners together with their counterparts from Australia and Japan to develop common recommendations for ontologies and metadata, together with the wider global bioimage data community. Through their joint forces and expertise, the foundingGIDE partners aim to make bioimage data FAIR and shareable across the globe, and to consolidate a network of researchers and communities that support an ecosystem of open image data sharing.
The project efforts will result in a consolidated landscape for image data sharing that will be built on community approved ontologies and metadata standards allowing interoperation between image data resources. This effort will serve as a blueprint for future national, regional and global initiatives to follow in order to incorporate into the data sharing ecosystem. Individual researchers will benefit from practical implementations, like the image search and analytics portals as well as guidelines for FAIR image data management.
Through its centrally coordinated efforts towards GIDE, foundingGIDE will provide a necessary framework for bioimage data to grow into a democratised resource enabling innovative research in Europe and across the globe.

From metabolites to signaling: glycolytic flux-control of embryo segmentation timing

Hidenobu Miyazawa , Jona Rada , Alexander Aulehla
EMBL Heidelberg

How metabolism impacts cell fate decisions during development and disease is a fundamental yet unresolved question. It is increasingly recognized that metabolism plays an important role beyond fueling biological processes, regulating gene expression and signal transduction. However, it remains a key challenge to distinguish a signaling role of metabolism from its canonical bioenergetic and biosynthetic functions. To address this challenge, we are exploring the role of glycolysis in mouse presomitic mesoderm (PSM) development, as it offers an opportunity to study the impact of metabolic perturbations on signaling dynamics in real time and in a quantitative manner. In this meeting, I will present our recent work demonstrating a modular organization of metabolic functions in PSM development. Our key findings include: 1) Unveiling a non-canonical signaling role of glycolytic metabolites that underlies the regulation of the segmentation clock period; 2) Demonstrating that this signaling role can be decoupled from its bioenergetic and biosynthetic roles. Lastly, I will also discuss about the potential mechanism and function of glycolytic metabolite signaling in development and beyond.

Generating spatial specificity in a dynamic stem cell system

Inés Hidalgo Prados
Centre for Organismal Studies

The shoot apical meristem (SAM) is a dynamic stem cell niche source of all aerial plant tissues. Signals promoting stem cell (SC) maintenance, cell differentiation and organ formation and growth need to be precisely coordinated. Whereas the WUSCHEL (WUS) – CLAVATA 3 (CLV3) feedback loop is important for SC homeostasis, auxin drives cell differentiation in the periphery. In the SAM, transcriptional response to auxin is mediated by AUXIN REPONSE FACTOR 5 (ARF5). It has been shown that WUS represses auxin signaling output in the central SCs (Ma et al., 2019). However, in flower primordia, WUS and auxin response coexist to drive floral organ development. The DNA-binding with One Zinc Finger (DOF) transcription factor (TF) OBFBINDING PROTEIN 1 (OBP1) is induced by auxin and is highly expressed in young flowerprimordia, where it co-localizes with WUS and high auxin response. Furthermore, OBP1 andWUS proteins interact. We hypothesize that OBP1 modulates WUS activity in the regulation of auxin output in flower primordia. Here, we show that expression of OBP1 under CLV3 leads to an enlarged SAM and an increased number of flowers. Furthermore, ectopic expression of OBP1 under the CLV3 promoter led to activation of auxin output in the central SCs. On the other hand, obp1 dof5.8 dof1.6 obp2 obp4 quintuple mutants showed a lower number of flowers compared to wild type. Lastly,

conversion of OBP1 into a dominant repressor revealed effects on WUS function in vegetative and floral meristems.

Cell organisation and disturbance-mapping using image activated cell-profiling (CODIAC)

Melanie Krause
European Molecular Biology Laboratory (EMBL), Genome Biology Unit

Protein localization and abundance is a key mechanism to regulate cell homeostasis. Furthermore, diseases are frequently associated with aberrant protein patters. However, high-throughput methods that monitor changes of many proteins at once are still lacking. Image enabled cell sorting has been demonstrated to add spatial and morphological information to classical cell sorting technology. We utilize ICS in combination with improved Cas12a PCR tagging to develop new ways to characterize proteome localization and expression levels within complex cellular pools. Applying ICS to fluorescently tagged protein libraries and machine learning, we have devised a novel way to assess cell organisation and to map perturbations using image activated cell-profiling (CODIAC). Using image-derived measurements from ICS, we group and isolate cells with fluorescently-tagged proteins of similar visual phenotypes. Sorting cell pools in their native state as well as upon chemical perturbation, we are able to identify changes in protein localization and abundance in a pooled fashion at a much faster pace than previously established. We expect this method to have broad applications in the field of high content screening for the identification of novel drug targets and various medical uses.

Adaptation and Reconfiguration of Morphogenesis Under Multi-Timescale Bending Stress

Soichiro Kato , Asako Shindo
Osaka University

The early developmental process of oviparous animals can be basically considered a "pre-determined" process, as it relies on a series of programmed gene expressions, cell movements, and organ constructions that occur in a timely manner using internally stored information and energy. However, “undetermined” processes also occur during late development, such as stochastically determined phenomena: e.g., embryonic posture inside egg

membrane, and random timing phenomena: e.g., escape movements from enemy or feeding behavior after hatching. How embryos respond to naturally occurred undetermined events and reconfigure the developmental process remains largely unknown. With this interest, we use Xenopus embryo as a model and focus on the relationship between organ morphogenesis and multi-timescale bending stresses: continuous left-right random bending in the egg membrane and fast fluctuating bending associated with swimming movement after hatching. For the continuous bending before hatching, we are focusing on symmetric organogenesis such as epidermis and somite. We recently found that the basement membrane of the epidermis at the hinge point forms a multi-layer like structure, suggesting that lateral epidermal tissue decrease surface area by forming new structure at the site. On the other hand, somite structure, although the shape is largely squashed, seems to be maintained under bending and its symmetry is recovered within short time after hatching. These data suggest that organs are thought to resist bending stresses in multiple ways. For the fast fluctuating bending after hatching, we are now making de novo tools to measure and perturb the swimming movement just after hatching stage to tackle the technically challenging question. In this symposium, we will discuss our research progress on these two themes.

Toward understanding the role of neuropeptide signaling in the regeneration of the sea anemone Nematostella vectensis

Sosuke Fujita , Lysander Blankenborg , Levin Riedel , Aissam Ikmi
EMBL Heidelberg, Developmental biology unit

Regeneration is the process of rebuilding lost body structures after damage. Comparative studies across highly regenerative animals have significantly contributed to our understanding of regeneration, with potential implications for regenerative medicine. In vertebrates, neuron-secreted growth factors such as FGF and BMP are crucial for proper regeneration. However, the role of the nervous system in regeneration and the evolutionary origins of its involvement remain poorly understood. Cnidarians, an early branching metazoan lineage encompassing jellyfish, corals, and anemones, offer valuable insights into the evolution of regeneration. Studies in Hydra suggested neurons play a limited role in regeneration, but this may not represent cnidarians as a whole. Moreover, neuropeptide signaling, a widespread form of neuronal communication, has been implicated in various developmental processes in cnidarians, including metamorphosis and oocyte maturation. However, its specific function in regeneration remains largely unexplored. Here, we investigate the role of neuropeptides in regeneration in the sea anemone Nematostella vectensis. A functional screen of 66 Nematostella neuropeptides

identified several hits that promote or restrict regeneration. At the cellular level, we found that specific neuropeptides induce localized patterns of cell proliferation in intact polyps. Strikingly, one neuropeptide demonstrated the remarkable ability to rescue animals artificially arrested in a dormant regenerative state. Our study offers a unique opportunity to study the neuronal control of regeneration and its evolutionary implications.

The evolution of gene regulation in mammalian cerebellum development

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Phenotypic innovations, such as the expansion of the mammalian brain, are thought to be driven by modifications in cell type- and time-specific gene regulatory networks (GRNs). However, the genetic underpinnings of the evolution of these networks are still largely unexplored. In this study, we combined previously published data with newly generated single-nucleus measurements of gene expression and chromatin accessibility in developing cerebellum from six mammalian species (human, bonobo, macaque, marmoset, mouse, and opossum), amassing approximately 758,000 single-nucleus profiles, to elucidate the genetic basis of the evolution of the mammalian cerebellum. Employing machine learning models, we inferred GRNs during cerebellum development and found that core GRNs are conserved across mammalian species. We developed a sequence-based deep learning model, DeepCeREvo, to decode the sequence grammar of cell type-specific cis-regulatory elements (CREs) across species. DeepCeREvo revealed that the regulatory code for cerebellar cell types has been conserved across mammals. Applying DeepCeREvo to orthologous genomic sequences from 240 mammalian species enabled us to reconstruct the evolutionary histories of human CREs. We found that evolutionarily novel CREs likely contributed to the divergence in gene expression observed between humans and other mammals. Furthermore, the DNA changes that are putatively responsible for the emergence of human-specific CREs have undergone positive selection in human populations. Finally,

we validated some of the predicted CRE innovations using enhancer reporter assays in primary cultures of mouse cerebellar granule cells. Our work delineates the genetic underpinnings of gene regulation evolution in developing cerebellum and offers insights into the molecular basis of mammalian phenotypic innovations.

Biological age dynamics in a renal development organoid model points to a rejuvenation phase and ground zero of aging

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Biological aging research focuses on embryonic development, challenging the notion that germ cells don't age. Evidence suggests a biological age reset during embryogenesis, with 'ground zero' identified where aging briefly reverses.
A rejuvenation phase in early embryogenesis has been revealed, where germ cells' biological age decreases despite metabolic activity. This process is linked to key developmental events and marks a new beginning for the organism.
Using novel clocks, biological age was tracked through iPSC differentiation, finding a rejuvenation phase during cell lineage specification. Different cell lineages showed varied patterns of biological age decrease and subsequent aging.
These findings support the 'ground zero' concept and establish iPSC-derived organoids as a model for studying biological age dynamics in early development. A system mimicking embryonic kidney development from human iPSCs was created. Analysis revealed two aging-related developmental processes with different patterns.
A comprehensive study across organoid cell lineages observed a consistent biological age decline at differentiation onset, followed by aging, indicating a common rejuvenation phase.
Further investigation into the molecular mechanisms underlying this "ground zero" phenomenon revealed significant mitochondrial involvement. Analysis of metabolic profiles and mitochondrial dynamics during the rejuvenation phase identified key regulatory pathways controlling biological age reset. Mining of public datasets using these novel insights is leading to the discovery of specific enhancers that regulate transcriptional programs associated with cellular rejuvenation. These findings have led to ongoing development of therapeutic compounds targeting these pathways.
This research successfully pinpointed cellular "ground zero" in vitro, bridging developmental and aging biology. It highlights potential for age manipulation

and rejuvenation in cellular models, offering a basis for interventions to reverse age-related decline and extend human healthspan. The study advances understanding of aging and rejuvenation at a cellular level, paving the way for future research and potential therapeutic applications.

Global BioImaging: Strengthening International Collaboration in Imaging Sciences

Yara Reis , Sophie Winter
EMBL

Global BioImaging is an international network dedicated to advancing imaging technologies and fostering global scientific collaboration. Since its establishment in 2016, the network has expanded to include partnerships with 13 national and regional organizations, including Euro-BioImaging in Europe and Advanced BioImaging Support (ABiS) in Japan. By connecting facility operators, technical staff, scientists, and policymakers across more than 60 countries, Global BioImaging enhances infrastructure accessibility, drives knowledge exchange, and supports the development of cutting-edge imaging solutions.
A key milestone in this collaboration was the Exchange of Experience (EoE2024) Conference, held in Okazaki, Japan, in partnership with ABiS. Focused on image data management and data repositories, the event saw significant growth in global participation, with 337 attendees from 48 countries, highlighting the increasing engagement from South-East Asia and beyond. This reflects the expanding scope of Global BioImaging's mission: to promote equitable access to imaging technologies and strengthen the research dialogue across borders.
At this conference, we present Global BioImaging's role in shaping the international bioimaging landscape and its ongoing efforts to foster scientific exchange between Europe, Japan, and the global research community. We welcome discussions on future collaborations and strategies to further integrate imaging infrastructures worldwide, ensuring a more connected and innovative research ecosystem.

Establishment and mechanism of action: effective chemical emasculation with trifluoromethanesulfonimide (TFMSA) in plant

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Hybridization plays an essential role in creating the diversity associated with genetic improvement of crops. To produce hybrids, emasculation methods such as hand removal of anthers, hot water treatment, genic or cytoplasmic male sterility, as well as chemical hybridizing agents (CHA) have long been studied and applied in many plant species. The ultimate aim of this study is to enable effective emasculation with CHA and automating cross pollination by insects. Trifluoromethanesulfonamide (TFMSA) has gained attention as a promising CHA in recent years and previously reported for induction of male sterility in maize and sorghum. We have applied TFMSA to plant species such as cowpea, pea , soybean, and other dicotyledonous species in total of 13 different species. Alexander staining showed 30 ml of 1000 mg/l TFMSA with two-time treatments at one-week interval, equivalent to 20.8 mg on the plant body, induced 99 % pollen sterility in cowpea. Two-time treatment of 10 ml of 125 – 250 mg/l TFMSA per plant induced non-functional pollen in diploid A. thaliana and N. benthamiana at two-time treatment of 10 ml of 250 –1000 mg/l per plant. Metabolomic analysis of A. thaliana flower buds and cowpea flower buds partially revealed the mechanism of action as the limitation of proline accumulation inside the anther. Our result will provide insight into the potential wide usage of TFMSA as an effective tool for production of hybrids in plants.

Functional analysis of intragenic cohesin binding sites with transcriptional regulation

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Cohesin complexes play an important role in the maintenance of three-dimensional structure and transcriptional regulation. Recent studies have reported that cohesin is involved in the formation of enhancer-promoter loops and in the transcription elongation by RNA polymerase II (Pol2). However, the function of some cohesin-binding sites, especially those that bind to intragenic regions, remains unclear. Recently, we generated ChIP-seq, RNA-seq, and Hi-C data from estrogen receptor (ER)-stimulated human MCF7 cells and performed a large-scale comparative analysis before and after ER stimulation. As a result, we identified 'decreased intragenic cohesin sites (DICs)' which are negatively

correlated with transcriptional regulation (Wang et al., Nat common, 2022). In addition, Data-driven analysis revealed that DICs are mainly located in intronic regions, and that they are classified into two major groups: cell-type common sites enriched in CTCF (HC-DICs) and cell-type specific sites lacking CTCF (LC-DICs). We also found that LC-DICs are strongly correlated with Pol2 and enhancer markers, whereas HD-DICs lack these characteristics and their function remains unknown. These findings suggest that DICs play an important role in gene expression. But we still have questions such as how DICs regulate gene expression and what is the function of HC-DICs. In this presentation, we would like to discuss the function of DICs along with the results of the multi-omics data analysis we performed to clarify this question.