Program

Sunday, July 2, 2017

14:00-18:00	**Registration** (Lobby of Nanyang Hotel)	
18:00-19:15	Welcome Reception (Chinese Dinning Hall)	
19:25-19:30	**Opening Remarks** (International Conference Hall)	

	Keynote Speeches (International Conference Hall)	
	Chairs: Quan Chen & Jiankang Liu	
19:30-20:15	**KS-1**, **Xiaodong Wang (NIBS, China)** *The Molecular Dissection of Mitochondrial Pathway of Apoptosis*	5
20:15-21:00	**KS-2**, **Gerald W. Dorn (Washington University in St. Louis, USA)** *Mitophagy, Mitochondrial Dynamics, and Heart Disease*	

Monday, July 3, 2017

	Session 1 Mitophagy (International Conference Hall)	
	Chairs: Koji Okamoto & Zhiyin Song	
08:30-09:00	**IL-1**, **Beth Levine (UT Southwestern, USA)** *Prohibitin 2, a Newly Identified Inner Mitochondrial Membrane Mitophagy Receptor*	6
09:00-09:30	**IL-2**, **Koji Okamoto (Osaka University, Japan)** *Upstream Events Regulating Atg32-Mediated Mitophagy*	
09:30–10:00	**IL-3**, **Zhiyin Song (Wuhan University, China)** *Sam50 Regulates PINK1-Parkin Mediated Mitophagy*	
10:00–10:30	**Group Photo and Coffee Break**	
10:30-11:00	**IL-4, Orian Shirihai (Boston University, USA)** *The Interplay Between Mitochondrial Biogenesis And Dynamics*	8
11:00-11:30	**IL-5, Quan Chen (Institute of Zoology, CAS, China)** *Mitochondrial FUNDC1 Regulates Selective Mitophagy and Proteostatic Stress Response*	9
11:30-11:45	**O-1, Hagai Abeliovich (Hebrew University of Jerusalem, Israel)** *Involvement of a Novel Phosophodegron-Like Selectivity Mechanism in Stationary Phase Mitophagy*	10
11:45-12:00	**O-2, Zhenji Gan (Nanjing University, China)** *Mitophagy Receptor Fundc1 in Skeletal Muscle Controls Systemic Glucose Homeostasis and Fat Metabolism*	11
12:20-14:00	Lunch	
12:30- 13:15	**Lunch Seminar by Seahorse and by Olympus**	12
13:30-15:30	**Poster session** (Room conference 8C)	
15:30- 16:00	Coffee Break	

Session 2 Ubiquitin and Mitochondrial Quality Control (International Conference Hall)		
Chairs: Ming Guo & Hui Jiang		
16:00-16:30	**IL-6, Agnieszka Chacinska (IIMBC, Polen)** *UPS-Dependent Degradation of Mitochondrial Precursor Proteins*	16
16:30-17:00	**IL-7, Hui Jiang (NIBS, China)** *Molecular Pathways of Mitochondrial Outer Membrane Protein Degradation*	17
17:00-17:30	**IL-8, Ming Guo (UCLA, USA)** *The PINK1/Parkin Pathway in Drosophila and Mammalian Systems*	18
17:30-17:45	**O-3, Chao Tong (Zhejiang University, China)** *Mitochondrial Protein Import Regulates Cytosolic Protein Homeostasis and Neuronal Integrity*	19
17:45-18:00	**O-4, Francois Ounton-Liger (ICM-Brain and Spine Institute, France)** *The PINK1/Parkin Pathway: a Regulatory Hub at The Intersection Between Mitochondrial Dysfunction and NLRP3 Inflammasome Signaling*	20
18:00-20:00	Dinner	

Tuesday, July 4, 2017

Session 3 Mitochondrial Proteases and Stress Responses (International Conference Hall)		
Chairs: Carolyin Suzuki & Shiori Sekine		
08:30-09:00	**IL-9, Luke Wiseman (TSRI, USA)** *Stress-Responsive Regulation of Mitochondria Proteostasis*	22
09:00-09:30	**IL-10, Jiankang Liu (Xi'an Jiaotong University, China)** *Control Mitochondrial Homeostasis with Mitochondrial Nutrients*	
09:30-09:45	**O-5, Hao Wu (Institute of Zoology, CAS, China)** *A Novel Axis Compring IRP1/Bcl-Xl Modulate Iron Stress Induced Mitophagy*	23
09:45-10:00	**O-6, Chunhong Chen (NHRI, Taiwan, China)** *Vesicle Trafficking Between Cytoplasm and Mitochondria: Potential Role of The Mitochondria-Associated ECM Protein*	23
10:00-10:30	Coffee break	
10:30-11:00	**IL-11, Shiori Sekine (NIH, USA)** *PARL-Mediated Cleavage of PGAM5 in Stress Responses*	24
11:00-11:30	**IL-12, Aleksandra Trifunovic (University of Cologne, Germany)** *Is Mammalian CLPP a Stress Protease?*	25
11:30-12:00	**IL-13, Carolyn Suzuki (UMDNJ, USA)** *Reprogramming Mitochondrial Bioenergetics by The AAA+ Lon Protease*	26
12:00-12:15	**O-7, Ye Tian (Institute of Genetics and Developmental Biology, CAS, China)** *Epigenetic Control of Mitochondrial Stress-Induced Longevity*	27
12:15-14:00	Lunch	
14:00-18:00	Free Discussion/Poster	

Wednesday, July 5, 2017

Session 4 Mitochondrial Dynamics and Biogenesis (International Conference Hall)		
Chairs: Nektarios Tavernarakis & Song Gao		
08:30-09:00	**IL-14, Luca Scorrano (University of Padua, Italy)** *Keeping Mitochondria in Shape: A Matter of Life and Death*	33
09:00-09:30	**IL-15, David Chan (CIT, USA)** *Control of Mitochondrial Degradation in The Early Embryo*	34
09:30-09:45	**O-8, Naotada Ishihara (Kurume University, Japan)** *Selective Mitohcondrial Fusion by Heterotypic Action Between OPA1 and Cardiolipin*	35
09:45-10:00	**O-9, Song Gao (Sun Yat-sen University, China)** *MFN1 Structures Reveal Nucleotide Triggered Dimerization Critical for Mitochondrial Fusion*	35
10:00-10:30	Coffee break	
10:30-11:00	**IL-16, Elena Rugarli (University of Cologne, Germany)** *A Post-Transcriptional Mechanism to Regulate Mitochondrial Function*	36
11:00-11:30	**IL-17, Nektarios Tavernarakis (IMBB, Greece)** *Coupling Mitophagy and Mitochondrial Biogenesis with Neurodegeneration During Ageing*	37
11:30-11:45	**O-10, Xingguo Liu (Guangzhou Institutes of Biomedicine and Health, CAS, China)** *Mitochondrial Remodeling in Somatic Cell Reprogramming*	38
11:45-12:00	**O-11, Chiara Calabrese (CECAD, Italy)** *Mitochondrial Quality Control Mechanisms Regulate TFEB*	38
12:00-14:00	Lunch	
Session 5 Mitochondrial Quality Control and Disease (International Conference Hall)		
Chairs: Thomas Langer & Chao Tong		
14:00-14:30	**IL-18, Chris Meisinger (University of Freiburg, Germany)** *The Mitochondrial Presequence Processing Machinery in Health and Disease*	33
14:30-15:00	**IL-19, Francesca Maltecca (The Vita-Salute San Raffaele University, Italy)** *Alteration of Mitochondrial Dynamics and Calcium Buffering in Cerebellar Degeneration*	34
15:00-15:15	**O-12, Bing Zhou (NIH, USA)** *Faciliataion of Axon Regeneration by Enhancing Mitochondrial Transport and Rescuing Energy Deficits*	35
15:15-15:30	**O-13, Patrice Petit (University Paris-Descartes, France)** *Cardiolipindeficienty Linked to Taz Gene Mutaion in the Barth Syndrome Affects Respriation Chain Function but Also Mitochondria Apoptosis and Mitophagy*	35
15:30-16:00	Coffee break	

16:00-16:30	**IL-20**, **Massimo Zeviani (MRC, UK)** *Mitochondrial Quality Control and Disease*	33
16:30-17:00	**IL-21, Zuhang Sheng (NIH, USA)** *Mechanism Removing Damaged Mitochondria from Axons*	34
17:00-17:30	**IL-22, Thomas Langer (University of Cologne, Germany)** *Proteolytic Control of Mitochondrial Function*	34
17:30-18:00	Panel Discussion, NCB/OCC Poster Award, and Closing Remarks	
18:15-20:00	Dinner	

Please note that every invited talk will be 30min including 10min discussion and every oral presentation will be 15min including 5min discussion.

KS-1

The molecular dissection of mitochondrial Pathway of apoptosis

Xian Jiang, Li Li, Hui Jiang, Zhiyuan Zhang, Xiaodong Wang
National Institute of Biological Sciences, Beijing
wangxiaodong@nibs.ac.cn

Ever since the signaling role of mitochondria in apoptosis was discovered 20 years ago, our lab has been trying to understand the molecular events inside mitochondria that lead to the release of apoptogenic proteins like cytochrome c and Smac from mitochondria to the cytosol during apoptosis. We first engineered a cell line based on human osteosarcoma U2OS cells in which pro-apoptotic proteins Bim or tBid could be inducibly expressed by adding doxycycline to the cultured cell media. The induction of Bim or tBid expression causes robust apoptosis in this cell line mediated by the Bim (or tBid) activated Bax and Bak, which form oligomers on the mitochondria, resulting in cytochrome c and Smac release. We first used this cell-based apoptosis system to verify the requirement of mitochondria cristae remodeling for apoptosis by demonstrating the cleavage of OPA1, a dynamin-like protein with multiple spliced isoforms that constitute protein complexes stabilizing cristae, is critical for apoptosis to occur in our system. We subsequently identified a mitochondrial inner membrane protease OMA1, as the protease responsible for cleaving OPA1 during apoptosis induction and OMA1 activation is downstream of Bax/Bak oligomerization. To further study how Bax/Bak activate OMA1 during apoptosis, we turned into chemical biological approach by screening a compound library with ~200,000 compounds for molecules that specifically block OMA1 activation but does not affect Bax/Bak oligomerization. We found one such a compound and after extensive medicinal chemistry modifications, were able to improve the cell protection efficacy of the compound to single digit nanomolar. The modified compound directly binds to succinate dehydrogenase subunit B (SDHB), a component of the mitochondrial electron transfer chain (ETC) complex II and prevents the disassembly of ETC complexes after apoptosis induction. We thus propose that Bim (and tBid) induced apoptosis starts with Bax/Bak activation and oligomerization, which not only changes the permeability of mitochondrial outer membrane, but also disrupts the mitochondrial ETC through an unknown mechanism. ETC disruption results in spikes of reactive oxygen species (ROS), which activates OMA1, leading to OPA1 cleavage, OPA1-containing complex disassembly, remodeling of cristae, and finally release of cytochrome c and other apoptogenic proteins located between outer and inner membrane of mitochondria.

These studies indicated that the disassembly of mitochondrial ETC, but not the change of permeability of outer membrane of mitochondrial, is the "point of no return" for apoptosis execution. For apoptosis to occur, there are two events need to happen: 1) outer membrane permeation mediated by oligomer Bax/Bak; and 2) disassembly of ETC that free cytochrome c from its electron transfer function.

One unintended result from the these studies is the finding of a mitochondrial protective chemical molecule. This molecule is able to keep cells alive and even continue proliferate even under mitochondrial stress conditions. Such a molecule may find usage in many situations including treatment of degenerative diseases.

KS-2
Mitophagy, Mitochondrial Dynamics, and Heart Disease

Gerald W. Dorn
Philip and Sima K Needleman Professor
Washington University in St. Louis School of Medicine
gdorn@wustl.edu

Mitochondrial dynamism and their selective elimination to maintain overall mitochondrial quality are functionally interconnected in cultured cells and linked to disease in Drosophila. Data supporting central roles for these processes in in vivo mammalian systems appear less straightforward. We have interrogated the interplay between mitophagy and mitochondrial fusion/fission in the mouse heart, an energy-dependent organ in which mitochondria are abundant, but hypodynamic, intrinsically fragmented, and which turn over slowly compared to other organs. Because the fusion protein Mfn2 is also an intermediate in mitochodrial PINK1-Parkin signaling, genetic deletion of Mfn2 (alone or in combination with Mfn1) interrupts mitophagy. In adult hearts this evokes mitochondrial dysfunction and rapidly lethal eccentric myocardial remodeling. By contrast, long-term genetic deletion of the fission protein Drp1 prevents sequestration of damaged mitochondrial elements in one daughter of non-replicative fission, impairing normal mitochondrial quality control, and ultimately provoking generalized mitophagic mitochondrial loss. The consequence in adult hearts is cardiomyocyte drop-out and rapidly lethal dilated cardiomyopathy. Remarkably, concomitant interruption of mitochondrial fusion and fission (Mfn1/Mfn2/Drp1 triple cardiac knockout) delays lethality, but ultimately produces mitochondrial hyper-accumulation, severe myocardial hypertrophy, and heart failure.

Novel genetic and pharmacological approaches have been developed to uncouple the fusion-promoting and Parkin receptor functions of Mfn2. Substitution of PINK1 phosphorylated T111 and S442 for non-phosphorylatable alanines (Mfn2 AA) renders Mfn2 a potent fusion protein, but dominant inhibitor of Parkin-mediated mitophagy. Expression of Mfn2 AA has no effects in adult hearts, suggesting that there are alternate mechanisms of mitochondrial quality control. In perinatal hearts Mfn2 AA disrupts the normal transition from fetal carbohydrate to adult fatty acid myocardial metabolism by interrupting developmental replacement of fetal by adult mitochondria. This uncovers an unexpected role for Mfn2-Parkin mitophagy signaling in metabolic transitions.

To modulate Mfn1- and Mfn2-mediated mitochondrial fusion without adversely impacting the Mfn2-Parkin mitophagy pathway, we have developed cell permeant mini-peptides and chemical peptidomimetics that direct mitofusins into fusion-favorable unfolded or –unfavorable folded conformations. These peptides and compounds are useful to correct pathology caused by pathological mitofusin loss or gain of function.

Session 1: Mitophagy

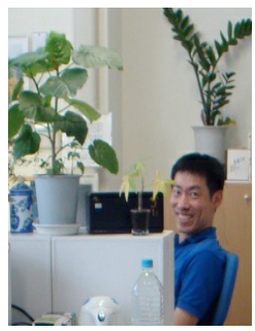
IL-1

Prohibitin 2, a Newly Identified Inner Mitochondrial Membrane Mitophagy Receptor

Beth Levine
Howard Hughes Medical Institute and University of Texas Southwestern Medical Center, Dallas, Texas USA
Beth.Levine@UTSouthwestern.edu

Mitophagy, the removal of unwanted or damaged mitochondria by autophagy is essential for key events in development, cellular homeostasis, and prevention of certain diseases and aging. Using an unbiased biochemical approach, we identified the inner mitochondrial membrane protein, prohibitin 2 (PHB2), as a crucial mitophagy receptor involved in targeting mitochondria for autophagic degradation. PHB2 binds the autophagosomal membrane-associated protein LC3 through an LC3-interaction region (LIR) domain upon mitochondrial depolarization and proteasome-dependent outer membrane rupture. PHB2 is required for Parkin-induced mitophagy in mammalian cells and for the clearance of paternal mitochondria after embryonic fertilization in C. elegans. These findings pinpoint a conserved mechanism of eukaryotic mitophagy and demonstrate a new function of prohibitin 2 that may underlie some of its physiological roles.

IL-2
Upstream events regulating Atg32-mediated mitophagy



Koji Okamoto
Graduate School of Frontier Biosciences, Osaka University
amano-kj@igakuken.or.jp

Mitophagy is an evolutionarily conserved catabolic process, acting as a mitochondrial quality and quantity control mechanism. In budding yeast, mitophagy is induced during prolonged respiratory growth via Atg32, a mitochondrial outer membrane protein acting as a receptor to recruit the autophagy machinery on the surface of mitochondria, although signaling pathways regulating degradation of mitochondria remain to be elucidated. We show here that mitophagy is strongly suppressed in respiring yeast cells lacking Iml1, Npr2, or Npr3, a component of the SEA subcomplex inhibiting TORC1 (SEACIT). By contrast, other autophagy processes in those mutants were slightly affected under the same conditions without amino acid deprivation. Rapamycin treatment markedly rescued mitochondrial degradation in cells lacking Npr2 at wild-type levels, suggesting that respiration-induced mitophagy requires SEACIT-dependent inactivation of TORC1. We also found that the TORC1-regulated phosphorylation status of Atg13, an activator of the Atg1 kinase, was not significantly altered in the absence of SEACIT. Notably, the atg13 npr2 double-null mutant exhibited more severe mitophagy defects compared with the atg13 or npr2 single-null mutant. Together, we propose that yeast cells at respiratory stationary phase undergo degradation of mitochondria through mitophagy-specific SEACIT upstream signal(s) and TORC1 downstream effector(s).

IL-3

Sam50 Regulates PINK1-Parkin mediated Mitophagy by Controlling PINK1 Stability and Mitochondrial Morphology

Fenglei Jian, Ziyin Song
Hubei Key Laboratory of Cell Homeostasis, College of Life Sciences, Wuhan University
songzy@whu.edu.cn

PINK1 (PTEN-induced putative kinase 1) and Parkin, genes associated with autosomal recessive early-onset Parkinsonism, mediate mitophagy, the clearance of dysfunctional mitochondria. This mitochondrial quality control process is also dependent on mitochondrial dynamics. However, the key molecules driving the change of mitochondrial shape and structure to promote or resist PINK1-Parkin mediated mitophagy are poorly identified. Here, we show that Sam50, the core component of sorting and assembly machinery (SAM) in mitochondrial outer membrane, is a critical regulator of mitochondrial dynamics and PINK1-Parkin mediated mitophagy. In response to Sam50 depletion, normal tubular mitochondria are first fragmented and subsequently altered to large spheres. Sam50 depletion resulted in mitochondrial dysfunction but maintained the normal mitochondrial membrane potential. Moreover, Sam50 deficiency promoted autophagy flux and induced PINK1-Parkin dependent mitophagy through the accumulation of PINK1 and Parkin on mitochondria. Furthermore, Sam50 facilitated PINK1 processing and degradation by interacting with PINK1, and Sam50 overexpression resisted to CCCP-induced PINK1 accumulation. Interestingly, during Sam50 depletion-induced mitophagy, mtDNAs accumulate in the spherical mitochondria and escape elimination. In C. elegans, we find that the Sam50 homolog gop-3 is required for the maintenances of mitochondrial morphology and mass in vivo. Our findings reveal that Sam50 directly links mitochondrial dynamics and mitophagy, and demonstrate that Sam50 downregulation is a novel driving force to initiate PINK1-Parkin dependent mitophagy.

IL-4

The interplay between mitochondrial biogenesis and dynamics

Orian Shirihai
David Geffen School of Medicine, UCLA
Shirihai@me.com

Mitochondria go through continuous cycles of selective fusion and fission, referred to as the "mitochondrial life cycle", to maintain the quality of its function (Twig et al, 2008, Twig et al 2010 ; Molina et al, 2009; Mouli et al, 2009). Changes in mitochondrial architecture can represent an adaptation of mitochondria to respire according to the bioenergetic needs of the cell (Liesa & Shirihai, 2013). Conditions requiring high mitochondrial ATP synthesis capacity and/or efficiency, such as limited nutrient availability, are associated with mitochondrial elongation (Gomes et al, 2011; reviewed in Liesa & Shirihai, 2013), while conditions of excess energy supply and relatively low ATP demand, such as b-cells exposed to excess nutrients, acutely induce mitochondrial fragmentation (Wikstrom et al, 2007). These changes enable more efficient engagement of the entire mitochondrial population of the cell in functions that favor a certain architecture. In this talk I will discuss examples where subpupolations of mitochondria within the same cell show different levels of mitochondrial dynamics, allowing them to remain distinct in content and function.

IL-5

Mitochondrial FUNDC1 regulates selective mitophagy and proteostatic stress response

Yanjun Li, and Quan Chen*
The State Key Laboratory of Membrane Biology, Chinese Academy of Sciences, Beijing 100101, China.
chenq@ioz.ac.cn

Mitophagy, a selective process that removes damaged or unwanted mitochondria, is critical for the maintenance of mitochondrial quality and quantity. Previously, we have revealed that FUNDC1, a mitochondrial outer-membrane protein, functions as a mitophagy receptor to mediate hypoxia-induced mitophagy. FUNDC1 harbors an LC-3 –interacting region (LIR) and interacts with LC-3 to mediate mitophagy both in cultured cell systems and in (patho-)physiological settings. We revealed that the reversible phosphorylation of FUNDC1 modulates its affinity with LC-3 for subsequent mitophagy. In an effort to understand the role of p62 in FUNDC1 mediated mitophagy, we found that FUNDC1 interacts with HSC70, a multifunctional cellular chaperone, to promote delivery of cytosolic client substrates via the TOM/TIM complexes to the mitochondrial matrix where some of them are degraded by the mitochondrial LONP1 protease. Accumulation of misfolded proteins in mitochondria promotes the formation of mitochondrion-associated protein aggregates (MAPAs), which are degraded by the FUNDC1-mediated mitophagy machinery. Our results reveal that mitochondria organize the complementary autophagic degradation of proteasomal clients, and have significant implications for understanding aging and aging-related diseases.

O-1

Involvement of a novel phosophodegron-like selectivity mechanism in stationary phase mitophagy

Hagai Abeliovich
Panagiota Kolitsida Hebrew University of Jerusalem
hagai.abeliovich@mail.huji.ac.il

Mitophagy, or the autophagic degradation of mitochondria, is an important housekeeping function of eukaryotic cells that prevents the accumulation of defective mitochondria due to oxidative damage and spontaneous mutation. The culling of defective mitochondria has been suggested to delay the onset of aging symptoms, and defects in mitophagy have been linked to late onset hereditary disorders such as Parkinson's disease and type II diabetes. We previously showed that different mitochondrial matrix proteins undergo mitophagy at different rates. An attractive hypothesis, supported by the existing data, is that mitophagy is preceded by segregation of defective mitochondrial components from undamaged ones, in a 'distillation' mechanism that leads to the selective turnover of defective compartments. We now demonstrate that dynamic mitochondrial matrix protein phosphorylation and dephosphorylation generate a segregation principle that could couple with mitochondrial fission and fusion to generate such a distillation process. The data indicate that structural determinants on a mitochondrial matrix protein can determine its mitophagic fate, independent of overall mitophagic flux, and that posttranslational modifications such as phosphorylation modify the function of these determinants. The results are consistent with a model wherein differences in protein-protein interactions between differentially phosphorylated proteins of the same species can drive a microscopic phase separation which, coupled with fusion-fission dynamics, could account for the observed selectivity.

O-2

Mitophagy receptor Fundc1 in skeletal muscle controls systemic glucose homeostasis and fat metabolism

Zhenji Gan
Model Animal Research Center of Nanjing University, Nanjing, China
ganzj@nju.edu.cn

Evidence is emerging that mitophagy serves as a major quality-control mechanism to prune damaged or dysfunctional mitochondria in mammalian cells. However, the in vivo physiological relevance of mitophagy remains poorly understood. Skeletal muscle is the major tissues of glucose and fatty acid consumption that heavily rely on mitochondrial metabolism, and, as such, mitochondrial function in skeletal muscle is a critical determinant of systemic metabolic homeostasis. Interestingly, muscle mitochondrial dysfunction as cause versus effect, or adaptive versus maladaptive, in the development of insulin resistance and glucose intolerance is debated. Whether and how the mitochondrial surveillance mechanism contributes to the divergent metabolic outcome of muscle mitochondrial stresses is unclear. In this study, we took advantage of the unique skeletal muscle metabolism system to explore the physiological consequences of skeletal muscle-specific Fundc1 loss-of-function on mitochondrial function and adaption to metabolic stress. Mice with Fundc1deficiency in skeletal muscle (Fundc1 mKO mice) exhibit a defect in LC3-mediated mitophagy, leading to impaired mitochondrial function and a marked reduced in exercise performance. Surprisingly, Fundc1 mKO mice are resistance to high-fat diet (HFD)-induced obesity and exhibit improved systemic insulin sensitivity and glucose tolerance. Thus our results demonstrate the pivotal role of mitophagy receptor FUNDC1 in regulating muscle mitochondrial function and systemic metabolic homeostasis, and highlight the potential therapeutic value of manipulating mitophagy in skeletal muscle to treat metabolic disorders.

Session 2: Ubiquitin and Mitochondrial Quality Control

IL-6

Regulation of cellular protein homeostasis by mitochondria

Agnieszka Chacinska
Centre of New Technologies, University of Warsaw
a.chacinska@cent.uw.edu.pl

Mitochondria must import the large majority of their proteome. We aim to understand consequences of defects in the mitochondrial protein import from the cellular perspective. Two main arms of the cellular response to mitochondrial protein import dysfunction include the inhibition of cytosolic translation and activation of the major protein degradation machinery, the proteasome. The stimulation of the proteasome is a direct response to the amount of mistargeted mitochondrial precursor proteins. To understand translational inhibition, in a collaborative study we performed a global, quantitative, and site-specific redox proteomic analysis to delineate the yeast redoxome up to a depth of more than 4,300 unique cysteine residues in over 2,200 proteins. Increased levels of intracellular ROS caused by the mitochondria serve as a signal to attenuate global protein synthesis. Mapping of redox-active thiols in proteins revealed ROS-sensitive sites in several components of the translation apparatus. We demonstrate that increased levels of intracellular ROS caused by dysfunctional mitochondria serve as a signal to attenuate global protein synthesis. Hence, we propose a universal mechanism that controls protein synthesis by inducing oxidative changes in the translation machinery.

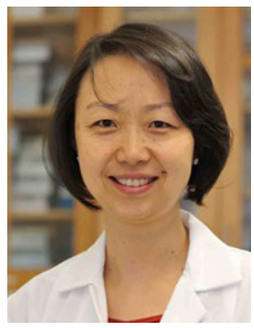
IL-7
Molecular pathways of mitochondrial outer membrane protein degradation

Hui Jiang, Xi Wu, Lanlan Li
National Institute of Biological Sciences, Beijing
jianghui@nibs.ac.cn

Mitochondrial proteostasis is critical for mitochondrial function. The current understanding is that mitochondrial proteome is under surveillance by two proteolysis systems: the ubiquitin-proteasome system degrades mitochondrial outer-membrane (MOM) proteins, and the AAA-proteases maintain the proteostasis of intra-mitochondrial compartments. The MOM associated protein turnover pathways have been poorly characterized. We recently systematically analyzed the turnover of MOM proteins in Saccharomyces cerevisiae, and identified a Doa1-Cdc48 complex that retrogradely translocates ubiquitinated MOM proteins to the cytoplasm for degradation. Unexpectedly, we also identified MOM proteins that are proteolyzed by the Yme1-Mgr1-Mgr3 i-AAA protease complex in mitochondrial inner-membrane. The Yme1 complex recognizes the inter-membrane space domains of the MOM substrates, and dislocates the MOM substrates into IMS for proteolysis. Our findings demonstrate that the MOM proteome is surveilled by mitochondrial and cytoplasmic quality control machineries in parallel.

IL-8

The PINK1/parkin pathway in Drosophila and mammalian systems



Ming Guo, M.D., Ph.D.
P. Gene & Elaine Smith Chair in Alzheimer's Disease Research
mingfly@ucla.edu

1) Mutations in PINK1 and parkin lead to autosomal recessive forms of Parkinson's disease. We showed that PINK1 and parkin function in a common pathway to regulate mitochondrial integrity and quality. This is accomplished in part through parkin-mediated degradation of Mitofusin, a protein that controls fusion. Overexpression of Mitofusin in Drosophila phenocopies cellular defects due to lack of PINK1 or parkin in Drosophila. Downregulation of mitofusin reverses phenotypes due to lack of PINK1 or parkin. Over the years, we have identified several suppressors of PINK1 and parkin null mutations, including Drp1, MUL1/MULAN/MAPL and VCP/p97. They also play conservative roles in mammalian cells and mice.

2) Mutations in VCP/p97 lead to autosomal dominant disease called inclusion body myopathy, Paget's disease and frontotemporal dementia (IBMPFD) and sporadic ALS. We have found that VCP disease mutations lead to disease through overactivation of VCP/p97, leading to excessive degradation of Mitofusin. Importantly, treatments of both Drosophila models and human patient fibroblasts with two different VCP/p97 inhibitors revert the pathology in these models. Therefore, VCP/p97 inhibition is an effective therapeutic strategy for IBMPFD.

3) Mitochondrial DNA (mtDNA) often exists in a state of heteroplasmy, in which mutant mtDNA co-exists in cells with wild-type mtDNA. High frequencies of pathogenic mtDNA result in maternally inherited diseases; somatically acquired mutations also accumulate over time and contribute to diseases of ageing that include neurodegeneration, metabolic disorders, cancer, heart disease and sarcopenia. Reducing heteroplasmy is therefore a therapeutic goal and in vivo models in post-mitotic tissues are needed to facilitate these studies. We have developed a transgene-based model of a heteroplasmic lethal mtDNA deletion in adult Drosophila post-mitotic tissue muscle. Stimulation of autophagy, activation of the PINK1/parkin pathway among others results in a selective decrease in mtDNA deletion (up to 95%). These results show that an adult post-mitotic tissue can be cleansed of a deleterious genome, suggesting that therapeutic removal of mutant mtDNA can be achieved.

O-3

Mitochondrial protein import regulates cytosol protein homeostasis and neuronal integrity

Wei Liu, Xiuying Duan, Chao Tong
Life Sciences Institute and Innovation Center for Cell Signaling Network, Zhejiang University, Hangzhou 310058, China
ctong@zju.edu.cn

Neurodegeneration is characterized by protein aggregate deposits and mitochondrial malfunction. It is not clear whether these two phenomena are intrinsically linked or not. RNAi screening data revealed that the loss of Tom40, a key subunit of the translocase of the outer mitochondrial membrane (TOM) complex, leads to abnormal accumulation of extremely large protein aggregates engulfed by autophagosome membranes in cytosol. Autophagy was induced in Tom40 RNAi tissues. However, the giant autophagosomes were often not sealed and they failed to fuse with lysosomes. The aggregates were ubiquitin-positive and accumulated large amounts of mitochondrial preproteins and cytosolic proteins such as Porin, Ref(2)P, and neuronal toxic protein Huntingtin polyQ. Several proteasome subunits were found in the aggregates, which may contribute to the reduction in proteasome activity in Tom40 RNAi tissues. The manipulation of the autophagy pathway in Tom40 RNAi tissues indicated that the giant autophagosome structures were formed through the fusion of small autophagosomes. The ectopic expression of Pink1 and Park greatly reduced aggregate formation in Tom40 RNAi tissues by increasing mitophagy, which however could be reversed by the inhibition of autophagy. In nerve tissues, the reduction in Tom40 activity leads to aggregate formation and neurodegeneration. Surprisingly, rather than diminishing the neurodegeneration phenotypes, the overexpression of Pink1 enhanced the neurodegeneration phenotypes. We proposed a model to demonstrate the mechanism by which mitochondrial protein import defects led to reduced proteasome activity and defective autophagy in the cytosol. This study also suggested that enhance mitophagy in neurons with mitochondrial defects might not be an effective approach to treat degeneration.

O-4

The PINK1/Parkin pathway: a regulatory hub at the intersection between mitochondrial dysfunction and NLRP3 inflammasome signaling

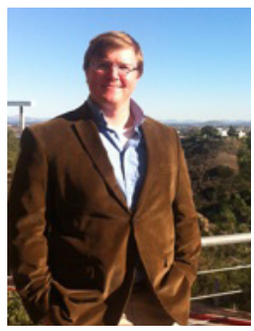
Francois MOUTON-LIGER [1, 2, 3, 4], Thibault ROSAZZA [1, 2, 3, 4], Julia SEPULVEDA [1, 2, 3, 4], Amélie IEANG[1, 2, 3, 4], Patrick MICHEL [1, 2, 3, 4], Jean-Christophe CORVOL [1, 2, 3, 4, 5], Olga CORTI 1, [2, 3, 4]
[1] Inserm, U1127, F-75013, Paris, France
[2]CNRS, UMR 7225, F-75013, Paris, France
[3]Sorbonne Universités, UPMC Univ. Paris 06, UMR S 1127, F-75013, Paris, France
[4] Institut du Cerveau et de la Moelle épinière, ICM, F-75013, Paris, France
[5]Assistance-publique Hôpitaux de Paris, Inserm, CIC-1422, Department of Neurology, Hôpital Pitié-Salpêtrière, F-75013, Paris, France
francois.mouton-liger@inserm.fr .

Mitochondrial dysfunction and neuroinflammation are central mechanisms in the pathogenesis of Parkinson's disease (PD). Mitochondria have emerged as key hubs in the build-up and modulation of innate immune responses. Loss of function mutations of the PARK2 and PARK6 genes, encoding the E3 ubiquitin protein ligase Parkin and the mitochondrial serine/threonine kinase PINK1, account for a large proportion of early-onset autosomal recessive PD cases PINK1 and Parkin regulate jointly mitochondrial quality control pathways that are dysfunctional in familial forms of PD, and they have also been linked to innate immunity. Recent studies provided evidence that overactivation of the NLRP3 inflammasome, a large signaling complex that senses and mounts reactions to infection and tissue damage, contributes to neurodegeneration in PD. Here, we show that PARK2 (or PARK6) deficiency leads to microglial overactivation characterized by exacerbated inflammasome signaling. Treatment with the bacterial endotoxin lipopolysaccharide or specific inflammasome activators (nigericin, ATP) led to increased intracellular NLRP3 levels and IL-1 β release in PARK2-or PARK6-deficient microglial cells compared to control cells. We demonstrate that NLRP3 and IL-1 β changes in PARK2-/- microglia is also observed at the mRNAs level and linked to regulation of the NF- κ B pathway. Overall, our results suggest that PINK1/Parkin deficiency and mitochondrial dysfunction work in concert to amplify NLRP3 activation in microglial cells through a new mechanism linking mitochondrial quality control and NF- κ B signaling.

Session 3: Mitochondrial proteases and stress responses

IL-9

Stress-Responsive Regulation of Mitochondria Proteostasis



Luke Wiseman
Department of Molecular Medicine, The Scripps Research Institute
La Jolla, CA 92037
wiseman@scripps.edu

Maintaining mitochondria protein homeostasis (or proteostasis) in response to cellular and organismal stress is critical to prevent pathologic imbalances in mitochondria function associated with etiologically-diverse human diseases. To confront this challenge, cells utilize stress-responsive signaling mechanisms to adapt the composition of mitochondria protein import, folding and proteolytic pathways responsible for regulating mitochondria proteostasis. The stress-responsive regulation of these pathways lead to profound changes in mitochondrial proteostasis capacity, morphology, and function that promote mitochondria biology during conditions of cellular stress. Here, I will discuss our recent efforts focused on defining the stress-responsive mechanisms responsible for regulating mitochondria proteostasis and how they integrate to protect the mitochondria proteome in response to diverse cellular insults. Through this discussion, I will describe how imbalances in mitochondria proteostasis regulation can contribute to the pathologic mitochondria dysfunction observed in human disease. In addition, I will describe new therapeutic opportunities to ameliorate pathologic defects in mitochondria biology by targeting the signaling pathways involved in mitochondria proteostasis regulation.

IL-10

Mitochondrial Homeostasis Control in Aging and Age-associated Diseases with Mitochondrial Nutrients

Jiankang Liu
Center for Mitochondrial Biology and Medicine, School of Life Science and Technology and Frontier Institute of Science and Technology, Xi'an Jiaotong University, Xi'an 710049, China
j.liu@mail.xjtu.edu.cn

It is the dream of human to live longer and healthier. More than 300 theories and hypotheses have been proposed for aging, however, the most accepted ones might be the "Free Radical Theory of Aging" and its derivative "Mitochondrial Theory of Aging", proposed by Dr. Denham Harman in last century. Mitochondria are the source and also the target of reactive oxygen species, therefore, mitochondrial dysfunction and redox imbalance have been suggested to play key roles in aging and age-associated diseases, including cardiovascular disease, Alzheimer's and Parkinson's Disease, Age-Associated Macular Degeneration, diabetes, and cancer. We have identified a group of nutrients, which could regulate mitochondrial homeostasis (biogenesis, mitophagy and dynamics) for improving mitochondrial function and promoting redox balance in aging and age-associated diseases. We have therefore designated these nutrients as "Mitochondrial Nutrients". In this presentation, I will summarize our recent progress in regulating mitochondrial homeostasis with a few of these Mitochondrial Nutrients. The in vitro and in vivo studies on these Mitochondrial Nutrients, especially their combinations, may help us to understand the significance of regulating mitochondrial homeostasis in delaying aging and preventing age-associated diseases.

O-5

A novel axis comprising IRP1 and Bcl-xL modulates iron stress-induced mitophagy

Hao Wu, Huifang Wei, Lei Liu, Quan Chen
The State Key Laboratory of Membrane Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China

Mitochondria are essential organelles for a multitude of cellular activities, including iron metabolism. Iron is imported into mitochondria for the synthesis of heme and iron-sulfur clusters (ISCs), both of which are iron-containing cofactors essential for the normal folding and structure of a series of proteins. It is widely recognized that iron deficiency can trigger Parkin-independent mitophagy, a major mechanism for mitochondrial quality control which involves selective recognition and engulfment of superfluous or damaged mitochondria by autophagosomes and their eventual degradation by lysosomes. However, the precise mechanism remains elusive. In this study, we found that the iron chelator DFO can activate the mitophagy receptor FUNDC1, and thus initiate FUNDC1-dependent mitophagy. Additionally, the iron sensor IRP1 is crucial for iron stress-induced mitophagy. Knockdown of IRP1 inhibits DFO-induced mitophagy. Mechanistically, we showed that the mRNA encoding Bcl-xL, a previously identified inhibitor of FUNDC1 dephosphorylation, FUNDC1 activation and FUNDC1-dependent mitophagy, is a novel target of IRP1. IRP1 binds to a newly characterized IRE localized in the 5'UTR of Bcl-xL mRNA, decreasing the translational efficiency of Bcl-xL, and thus leading to FUNDC1 dephosphorylation and activation during iron stress. In conclusion, we uncovered a novel IRP1-Bcl-xL signaling axis which modulates iron stress-induced mitophagy, and we demonstrated the crucial role of this induced mitophagy in mitochondrial quality control in response to iron stress.

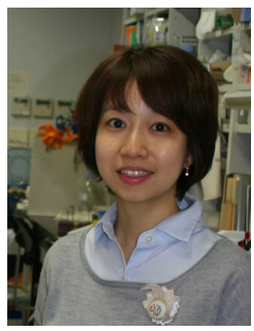
O-6

Vesicles trafficking between cytoplasm and mitochondria: Potential role of the mitochondria-associated ECM protein

CHUN-HONG CHEN [1], PO-LIN CHEN[2]
[1] Institute of Molecular and Genomic Medicine, National Health Research Institutes.
[2] Institute of Cellular and Molecular Biology, National Taiwan University
chunhong@gmail.com

Vesicles trafficking is a molecular process through which vesicles containing various types of materials are transported within cells, which includes the release of peroxisomes from mitochondria. Multiple Rab proteins that play important roles in vesicles trafficking. We investigated whether cytoplasmic vesicles are transported into the mitochondria in transgenic Drosophila, as well as the potential mechanism by which the vesicle uptake occurs. We found that the overexpression of the mitochondria-associated protein, here we named it as Enlarged and Clustering Mitochondria (ECM), caused mitochondrial enlargement and clustering, and there are double-membrane vesicles within mitochondria which concurrently with aging phenotype. In this report , we proposed that the enlargement of mitochondria in cells overexpressing ECM is caused by the uptake these double-membrane vesicles. The results showed that ectopically expressed cytosolic GFP localized to the enlarged mitochondria of myocytes overexpressing ECM. Immunogold labeling showed the cytosolic GFP was located within the vesicles of enlarged mitochondria, and GFP also presented in the mitochondria fraction of cells overexpressing ECM. In order to examine which Rab protein involve in this process, overexpression of the full-length GTPase, Rab32, and the separate overexpression of the constitutive form of Rab32 enhanced the ECM-induced mitochondrial phenotype, whereas expression of the dominant negative form of Rab32 partially suppressed the ECM-induced mitochondrial phenotype and GFP level within mitochondria overexpressing ECM. In addition, the autophagy markers, Atg8 and Ref(2)P, accumulated in cells overexpressing ECM, indicating that an incomplete activation of autophagy had occurred. The interaction between ECM mediates vesicle transport from the cytoplasm into mitochondria, and the ECM-induced enlargement of mitochondria and autophagic processes will be discussed.

IL-11
PARL-mediated cleavage of PGAM5 in stress responses



Shiori Sekine
NINDS, NIH
shiori.sekine@nih.gov

Phosphoglycerate mutase 5 (PGAM5) is a Ser/Thr protein phosphatase that resides in mitochondria. Unlike the classical Ser/Thr protein phosphatase, PGAM5 uses histidine residue that is well-conserved throughout several species form C. elegans to human, as a catalytic center for exhibiting its protein phosphatase activity [1]. Under steady state, PGAM5 is localized in the inner mitochondrial membrane (IMM) through its Nterminal transmembrane domain facing its phosphatase domain to the intramembrane space. We previously identified that PGAM5 is cleaved within its transmembrane domain by PARL (presenilin-associated rhomboid-like), an IMM-resident rhomboid protease [2]. PARL is also known to cleave PINK1, an IMM-resident Ser/Thr protein kinase responsible for autosomal-recessive Parkinoson's disease. Interestingly, while PARL-mediated cleavage of PINK1 constitutively occurs in healthy mitochondria that maintain intact mitochondrial membrane potential, PGAM5 cleavage by PARL is strongly induced in damaged mitochondria that lose mitochondrial membrane potential. Recent studies indicate that cleaved PGAM5 is released into cytosol in response to apoptosis-inducing stimuli and acts as an apoptosis promoter through inhibiting IAP-family proteins [3], suggesting that PARL-mediated cleavage of PGAM5 has important roles in cellular stress responses. In this talk, I would like to update the mechanisms of PARL-mediated PGAM5 cleavage, and also introduce our recent findings on the phenotypes of PGAM5 knockout mice under the metabolic stress conditions [4].

[1] Takeda K, et al. Proc Natl Acad Sci USA. 2009;106:12301-12305.

[2] Sekine S, et al. J Biol Chem. 2012;287:34635-34645.

[3] Zhuang M, et al. Mol Cell. 2013;49:273-282.

[4] Sekine S, Yao A, et al. EBiomedicine. 2016; 5:82-92.

IL-12
Is mammalian CLPP a stress protease?

Aleksandra Trifunovic
Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), and Institute for Mitochondrial Diseases and Aging, Medical Faculty, University of Cologne, D-50931 Cologne, Germany
aleksandra.trifunovic@uk-koeln.de

CLPP is highly conserved from bacteria to humans. In concert with CLPX, an ATP-dependent protein unfoldase, CLPP forms the bulky proteasome-like machinery (ClpXP) residing in the matrix of animal cell mitochondria. We have recently presented evidences that mammalian CLPP has an essential role in determining the performance of mitochondrial protein synthesis. In heart mitochondria CLPP impacts the translation by regulating the level of mitoribosome assembly. Consequently, CLPP deficiency results in a mild respiratory chain defect with prevailing Complex I dysfunction. Through a proteomic approach and the use of a catalytically inactive CLPP we produced the first comprehensive list of possible mammalian ClpXP substrates involved in the regulation of mitochondrial translation, oxidative phosphorylation and a number of metabolic pathways. We further show that the defect in mitoribosomal assembly in heart is the consequence of ERAL1 accumulation, a putative 12S rRNA chaperone and novel ClpXP substrate. Our data suggested that the timely removal of ERAL1 from the small ribosomal subunit is essential for the efficient maturation of mitoribosomes and a normal rate of mitochondrial translation. To further dissect the role of CLPXP protease in regulation of OXPHOS biogenesis we have analyzed the respiratory chain components revealed as CLPP substrates in our proteomic screen. We showed that CLPXP protease is required for turnover of N-module part of matrix-exposed peripheral arm of Complex I and upon CLPP dysfunction an entire catalytically active N-module accumulates in a mitochondrial matrix. Our data indicate that CLPP dependent N-module accumulation is further exaggerated upon metabolically induced OXPHOS remodeling. Furthermore, CLPXP protease is indispensable to clearing removal of Complex I N-module upon respiratory chain defect in vivo. Our findings suggest that CLPP plays an unforeseen role in remodeling of Complex I upon metabolic fuel switch and CLPXP activity can modulate Complex I stability upon diseases-related respiratory chain dysfunction.

IL-13
Reprogramming mitochondrial bioenergetics by the AAA+ Lon protease

Carolyn Suzuki [1] , Sundararajan Venkatesh [1] , Ashutosh Pandey [1] , Jae Lee [1] , Min Li [1] , Eman Rashed [1] , Toshiro Saito [2] , Junichi Sadoshima [2] , Graeme Nimmo [3] , Grace Yoon [3]

[1]Rutgers-New Jersey Medical School, USA, Dept. of Microbiology, Biochemistry & Molecular Genetics, Rutgers-New Jersey Medical School, USA

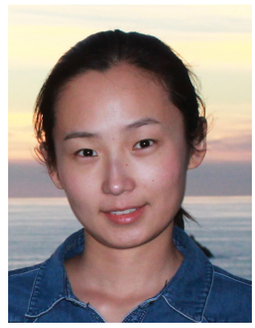
[2]Cell Biology and Molecular Medicine, Rutgers-New Jersey Medical School, USA

[3]Division of Clinical and Metabolic Genetics, Department of Pediatrics, The Hospital for Sick Children, Toronto, Canada

suzukick@njms.rutgers.edu

The mitochondrial Lon protease is a major regulator of mitochondrial proteostasis, respiration and metabolic flux, which is essential during embryogenesis and throughout development. Lon degrades misfolded and damaged proteins, as well as key rate-limiting proteins involved in mitochondrial DNA maintenance and expression, the electron transport chain and intermediate metabolism. Pathogenic mutations in the LONP1 gene encoding mitochondrial Lon have been linked to two rare developmental disorders. The first disease to be identified is CODAS syndrome, which is characterized by cerebral, ocular, dental, auricular and skeletal anomalies. Affected individuals are from diverse ethnic backgrounds- Amish-Swiss, Menonite-German, mixed European, Turkish, Korean and Brazilian, carrying either homozygous or compound heterozygous LONP1 mutations. Initial characterization of the Amish allele c.2161C>G (p.Arg721Gly), showed that the LonR721G mutation increases the thermosensitivity of the homo-hexameric protease, and substantially reduces its ATPase and protease activities and bioenergetic reserve capacity. In addition to CODAS syndrome, we recently identified a novel homozygous LONP1 mutation c.2282C>T (p.Pro761Leu) in 2 Afghani siblings presenting with profound neuromuscular dysfunction and global developmental delays with stepwise regression. These clinical phenotypes are distinctly different from those associated with CODAS syndrome. The Lon-P761L mutation also destabilizes the holoenzyme, increasing its thermosensitivity, substantially reducing its enzymatic activities and spare respiratory capacity. In both siblings, increased intracellular lactate:pyruvate ratios are observed, in addition to reduced native pyruvate dehydrogenase activity, and reduced Complex I and IV activity. Carbon substrate utilization was altered in the Siblings as compared to their Mother. Experiments are underway to delineate the physiological and mechanistic differences, which underlie this novel disease and CODAS syndrome, to elucidate the diverse tissue-specific dysfunctions resulting from mutations in the mitochondrial Lon protease.

O-7
Epigenetic control of mitochondrial stress-induced longevity



Ye Tian
Institute of Genetics and Developmental Biology, Chinese Academy of Sciences
ytian@genetics.ac.cn

Environmental stresses and aging process often perturb mitochondrial function and trigger mitochondrial stress response through the upregulation of an array of protective genes. The mitochondrial stresses occurred at a particular time during development can be sensed and communicated across tissues, eliciting a life-extending change across a lifetime. We find that mitochondrial stress during development causes widespread changes in chromatin structure through histone H3K9 di-methylation marks traditionally associated with gene silencing. Mitochondrial stress response activation requires the di-methylation of histone H3K9 through the activity of the histone methyltransferase met-2 and the nuclear co-factor lin-65. While globally the chromatin becomes silenced by these marks, remaining portions of the chromatin open up, at which point the binding of canonical stress responsive factors such as DVE-1 occurs. Thus, a metabolic stress response is established and propagated into adulthood of animals through specific epigenetic modifications that allow for selective gene expression and lifespan extension. Reference: Tian, Y., Garcia, G., Bian, Q., Steffen, K., Joe, Larry., Wolff, S., Meyer, B.J., and Dillin, A.* (2016) Mitochondrial stress induces chromatin reorganization to promote longevity and UPRmt. Cell 165, 197-208

Session 4: Mitochondrial dynamics and biogenesis

IL-14

Keeping mitochondria in shape: a matter of life and death

Luca Scorrano

IL-15
Control of mitochondrial degradation in the early embryo

Rebecca Rojanksy, Moon-Yong Cha, David Chan
Division of Biology and Biological Engineering, California Institute of Technology
Pasadena, CA 91125, USA
dchan@caltech.edu

Maternal inheritance is a signature feature of mitochondria. Sperm contribute mitochondria to the zygotes, but these paternal mitochondria are eliminated, so that inheritance of mitochondria and their DNA is uniparental. A role for mitophagy (the autophagic degradation of mitochondria) in the elimination of paternal mitochondria has been shown in early nematode embryos. It is not clear if a similar mechanism exists in mammalian embryos. To study the elimination of paternal mitochondria, we tracked the fate of sperm mitochondria in mouse embryos after fertilization. PhAM mice ubiquitously express mito-Dendra2, a photo-convertible fluorophore localized to the mitochondrial matrix. When PhAM males are mated to normal females, the resulting embryos contain fluorescently labeled mitochondria that can be tracked by fluorescence microscopy. We found that paternal mitochondria do not fuse with other mitochondria, eventually lose their membrane potential, and are selectively eliminated from the embryo by 80 hours after fertilization. In contrast, maternal mitochondria are stable and fusion-active. To test the role of specific genes in paternal mitochondria elimination, we used lentivirus encoding shRNA to disrupt the function of genes in the early embryo.

O-8

Selective mitochondrial fusion by heterotypic action between OPA1 and cardiolipin

Naotada Ishihara
Department of Protein Biochemistry, Institute of Life Science, Kurume University, Kurume, Japan
ishihara_naotada@kurume-u.ac.jp

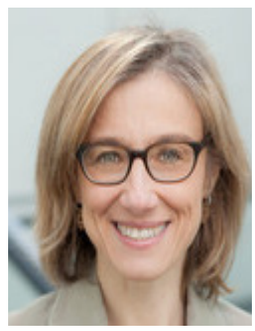
Optic atrophy 1 (OPA1) is an essential GTPase protein for mitochondrial inner membrane (IM) fusion. Under mitochondria-stress conditions, IM-anchored L-OPA1 is proteolytically cleaved to form peripheral S-OPA1, leading to selection of damaged mitochondria for the quality control (Ishihara N. et al, EMBO J. 2006). However, the molecular details of the selective mitochondrial fusion were less well understood. Here we reconstituted an in vitro membrane fusion reaction using purified human L-OPA1 and S-OPA1 expressed in silkworm, and found that L-OPA1 and the mitochondria-specific phospholipid cardiolipin (CL) cooperate in the selective mitochondrial fusion. GTP-independent membrane tethering through L-OPA1 and CL primes the subsequent GTP-hydrolysis-dependent fusion, as the most minimal intracellular membrane fusion machinery. These data unveil a novel role of CL in mitochondrial quality control, which modulates OPA1-dependent mitochondrial membrane fusion.

O-9
Structures of human mitofusin and hints in mitochondrial tethering

Song Gao [1], Yu-Lu Cao [1], Shuxia Meng[2], David Chan [2]
[1]State Key Laboratory of Oncology in South China, Sun Yat-sen University Cancer Center
[2]Division of Biology and Biological Engineering, California Institute of Technology
gaosong@sysucc.org.cn

Mitochondria are double-membrane organelles with varying shapes influenced by metabolic conditions, developmental stage, and environmental stimuli. Their dynamic morphology is realized through regulated and balanced fusion and fission processes. Fusion is crucial for the health and physiological functions of mitochondria, including complementation of damaged mitochondrial DNAs and maintenance of membrane potential. Mitofusins (MFNs) are dynamin-related GTPases essential for mitochondrial fusion. They are embedded in the mitochondrial outer membrane and thought to fuse adjacent mitochondria via concerted oligomerization and GTP hydrolysis. However, the molecular mechanisms behind this process remains elusive. We determined crystal structures of engineered human MFN1 containing the GTPase domain and a helical domain in different stages of GTP hydrolysis. The helical domain is composed of elements from widely dispersed sequence regions of MFN1 and resembles the neck of the bacterial dynamin-like protein. The structures reveal unique features of its catalytic machinery and explain how GTP binding induces conformational changes to promote G domain dimerization in the transition state. Disruption of G domain dimerization abolishes the fusogenic activity of MFN1. Moreover, a conserved aspartate trigger was found in MFN1 to affect mitochondrial elongation, likely through a GTP-loading-dependent domain rearrangement. Based on these results, we propose a mechanistic model for MFN1-mediated mitochondrial tethering. Our study provides important insights in the molecular basis of mitochondrial fusion.

IL-17
A post-transcriptional mechanism to regulate mitochondrial function



Elena Rugarli
Institute for Genetics, University of Cologne, Cologne, Germany
Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany.
Elena.Rugarli@uni-koeln.de

Mitochondria contain more than 1,500 proteins, most of which are encoded in the nucleus, translated in the cytosol, and subsequently imported into the mitochondrion. It is still poorly understood whether expression of mitochondrial proteins in mammals is regulated post-transcriptionally, by controlling stability, translation rate or localization of their transcripts. CLUH is an evolutionary conserved protein, the deletion of which leads to mitochondrial clustering next to the nucleus in several species. Recently, we demonstrated that CLUH is an RNA-binding protein that binds specifically a subset of mRNAs encoding mitochondrial proteins. CLUH target mRNAs are involved in many metabolic pathways involved in energy conversion, such as OXPHOS, TCA cycle, fatty acid oxidation and amino acid degradation. By modeling CLUH deficiency in the mouse, we found that CLUH is essential to reshape the mitochondrial proteome to face conditions of nutrient deprivation, such as the fetal-neonatal transition or starvation.

IL-17
Coupling mitophagy and mitochondrial biogenesis with neurodegeneration during ageing

Nektarios Tavernarakis
Foundation for Research and Technology-Hellas
tavernarakis@imbb.forth.gr

Mitochondrial function impinges on several signalling pathways modulating cellular metabolism, cell survival and healthspan. Maintenance of mitochondrial homeostasis requires both generation of new, and elimination of dysfunctional mitochondria. Impaired mitochondrial content homeostasis is a common characteristic of ageing and several human pathophysiological conditions, highlighting the pivotal role of the coordination between mitochondrial biogenesis and mitophagy. However, the cellular and molecular underpinnings of the relevant mechanisms remain obscure. We found that DCT-1, the Caenorhabditis elegans homolog of mammalian BNIP3 and BNIP3L/NIX, is a key mediator of mitophagy, promoting longevity under stress. DCT-1 acts downstream of the PINK-1/Parkin pathway and is ubiquitinated upon mitophagyinducing conditions to mediate the removal of damaged mitochondria. Accumulation of damaged mitochondria triggers SKN-1 activation, which initiates a bipartite retrograde signaling pathway stimulating the coordinated induction of both mitochondrial biogenesis and mitophagy genes. Our results unravel a homeostatic feedback loop that allows cells to adjust their mitochondrial population in response to environmental and intracellular cues. Age-dependent decline of mitophagy both inhibits removal of dysfunctional or superfluous mitochondria and impairs mitochondrial biogenesis resulting in progressive mitochondrial accretion and consequently, deterioration of cell function.

O-10
Mitochondrial remodeling in somatic cell reprogramming

Xingguo Liu
Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, China
liu_xingguo@gibh.ac.cn

Reprogramming of mouse embryonic fibroblasts (MEFs) into induced pluripotent stem cells (iPSCs) by Yamanaka factors (Sox2, Klf4, Oct4 and c-Myc) has been recognized as a fundamental breakthrough in biology and medicine. iPSCs have less and immature mitochondria than somatic cells and mainly rely on glycolysis for energy source. During somatic cell reprogramming, somatic mitochondria and other organelles get remodeled. However, events of mitochondria remodeling during somatic cell reprogramming have not been extensively explored. Here we show that mitochondrial flashes (mitoflashes), recently identified spontaneous bursts of mitochondrial superoxide and pH signaling, is transiently activated and mitochondrial mass is decreased by a novel function of mitophagy in somatic cell reprogramming. The frequency of mitoflashes transiently increases, accompanied by flash amplitude reduction, during the early stages of reprogramming. This transient activation of mitoflashes at the early stage enhances reprogramming, whereas sustained activation impairs reprogramming. The reprogramming-promoting function of mitoflashes occurs via the upregulation of Nanog expression that is associated with decreases in the methylation status of the Nanog promoter through Tet2 occupancy. Both SKO (Sox2, Klf4 and Oct4) and SKOM (SKO plus c-Myc) reprogramming lead to decreased mitochondrial mass but with different kinetics and by divergent pathways. Rapid, cMYC-induced cell proliferation may function as the main driver of mitochondrial decrease in SKOM. In SKO reprogramming, however, mitochondrial mass initially increases and subsequently decreases via mitophagy. This mitophagy is dependent on the mitochondrial outer membrane receptor BNIP3L/NIX but not on mitochondrial membrane potential ($\Delta\Psi m$) dissipation, and this SKO-induced mitophagy functions in an important role during the reprogramming process. Our study reveals the rule and the role of mitochondrial remodeling in somatic cell reprogramming

O-11
Mitochondrial quality control mechanisms regulate TFEB

Chiara Calabrese [1,2], Raja Ganesan [1,2], Saray Gutierrez [1,2], Hendrik Nolte [1], Joanna Stepek [1,2], Nirmal Robinson [1,2]
[1]Cologne Cluster of Excellence in Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany.
[2] Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Cologne, Germany.
ccalabre@smail.uni-koeln.de

The helix-loop-helix leucine zipper transcription factor, TFEB, was identified to transcriptionally regulate lysosome biogenesis and autophagy. Our preliminary studies in Salmonella Typhimurium (S. Typhimurium)-infected cells suggest that TFEB possibly has a cytosolic function in addition to its well-characterized role as a transcription factor. Therefore, we aimed to dissect the functions of TFEB in the cytosol and nucleus. An unbiased proteomics approach strikingly revealed that TFEB interacts with several mitochondrial proteins, which is lost when infected with S. Typhimurium. Microscopical and biochemical examinations further confirmed that TFEB localizes in mitochondria. Furthermore, mutation of a TOMM20 binding motif prevented its mitochondrial translocation. Surprisingly, inhibition of mTOR, which is known to induce nuclear localization of TFEB, was also able to increase the translocation of TFEB into mitochondria compared to untreated cells. Since we observed a decline in mitochondrial TFEB upon S. Typhimurium infection, we hypothesized that TFEB could traffic into the mitochondria for degradation. In order to investigate how TFEB could be degraded in the mitochondria, we knocked down ClpX a component of ClpXP and LonP two of the major mitochondrial proteases using specific siRNAs and investigated the expression of TFEB. Surprisingly, the knockdown of LonP but not ClpX resulted in an increase in TFEB, suggesting that its degradation within the mitochondria is LonP-dependent. Taken together, our results clearly show that TFEB is regulated by a mitochondrial quality control mechanism upon infection and stress.

Session 5: Mitochondrial quality control and disease

IL-18

The mitochondrial presequence processing machinery in health and disease

Chris Meisinger
Institute of Biochemistry and Molecularbiology and BIOSS (Centre for Biological Signalling Studies), Freiburg University, Germany

Mitochondria have to import most of their proteins in order to fulfill a multitude of metabolic functions. Sophisticated import machineries mediate targeting and translocation of preproteins from the cytosol and their subsequent sorting into the suborganellar destination. For a long time the mode of action of these machineries has been considered as a static and constitutively active process. We found that the preprotein import machinery is highly phosphorylated and targeted by several cytosolic and mitochondrial protein kinases. They dynamically regulate organellar protein import to adjust the specific requirements of mitochondria to changes in metabolic conditions. Moreover, we uncovered the first link between cell cycle regulation and the mitochondrial import machinery.

IL-19
Alteration of mitochondrial dynamics and calcium buffering in cerebellar degeneration

Francesca Maltecca
Vita-Salute San Raffaele University and Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Milan, Italy
maltecca.francesca@hsr.it

Degeneration of Purkinje cells (PCs) is the most common neuropathological feature of cerebellar ataxias, neurodegenerative conditions characterized by altered motor coordination and balance. PCs are neurons with unique features: they are characterized by a large soma and extensive dendritic trees receiving massive glutamate excitatory stimuli, and therefore exposed to high cytosolic calcium concentrations. Synapses of PC dendrites need a more precise control of calcium homeostasis compared to other neurons, implying higher levels of ATP and higher calcium buffering power. These features suggest that proper mitochondrial functionality and distribution to microdomains of large ion fluxes represent crucial issues in PCs. Indeed, mitochondria not only provide ATP to active calcium clearance systems at the plasma membrane, but also exert themselves a fine shaping of calcium signals by accumulating calcium into the matrix. Experimental mouse models in which MFN2 or DRP1 are specifically ablated in PCs develop defects in motor coordination and PC degeneration associated to aberrant mitochondrial ultrastructure and altered mitochondrial distribution. We demonstrated the critical dependence of PCs on mitochondrial dynamics in physiopathological conditions, i.e. in inherited forms of cerebellar ataxia. Spinocerebellar ataxia type 28 (SCA28) is caused by mutations of the mitochondrial protease AFG3L2. We found that in both SCA28 mouse model and SCA28 patient fibroblasts mutant mitochondria ineffectively buffer the evoked calcium peaks. We proved in primary PCs that this defect enhances cytoplasmic calcium concentration and finally triggers PC degeneration. Deregulated calcium homeostasis is caused by the negative synergism between mitochondrial depolarization and altered trafficking of the organelles to PC dendrites, due to excessive mitochondrial fragmentation. Proving this mechanism, we completely recovered the ataxic phenotype of SCA28 mice by genetically reducing the metabotropic glutamate receptors mGluR1, and thus decreasing calcium influx in PCs. The same result has been successfully replicated by administration of an off-label therapy favoring the synaptic glutamate clearance. We recently found that in another form of cereballar ataxia, ARSACS, in which mitochondria show excessive elongation, mitochondria are retained in PC soma and poorly trafficked to PC dendrites. Taken tohether, the findings highlight defective mitochondrial dynamics as a common mitochondrial pathway to PC degeneration in cerebellar ataxias.

O-12
Facilitation of axon regeneration by enhancing mitochondrial transport and rescuing energy deficits

Bing Zhou[1], Panpan Yu[2], Mei-Yao Lin[1], Tao Sun[1], Yanmin Chen[1], Zu-Hang Sheng[1]
[1]Synaptic Functions Section, The Porter Neuroscience Research Center, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892
[2]Guangdong–Hong Kong–Macau Institute of CNS Regeneration, Ministry of Education Joint International Research Laboratory of CNS Regeneration, Jinan University, Guangzhou 510632, China
zhoub2@hotmail.com

Although neuronal regeneration is a highly energy-demanding process, axonal mitochondrial transport progressively declines with maturation. Mature neurons typically fail to regenerate after injury, thus raising a fundamental question as to whether mitochondrial transport is necessary to meet enhanced metabolic requirements during regeneration. Here, we reveal that reduced mitochondrial motility and energy deficits in injured axons are intrinsic mechanisms controlling regrowth in mature neurons. Axotomy induces acute mitochondrial depolarization and ATP depletion in injured axons. Thus, mature neuron-associated increases in mitochondria-anchoring protein syntaphilin (SNPH) and decreases in mitochondrial transport cause local energy deficits. Strikingly, enhancing mitochondrial transport via genetic manipulation facilitates regenerative capacity by replenishing healthy mitochondria in injured axons, thereby rescuing energy deficits. An in vivo sciatic nerve crush study further shows that enhanced mitochondrial transport in snph knockout mice accelerates axon regeneration. Understanding deficits in mitochondrial trafficking and energy supply in injured axons of mature neurons benefits development of new strategies to stimulate axon regeneration.

O-13

Cardiolipin deficiency linked to Taz gene mutation in the Barth syndrome affects respiratory chain function but also mitochondrial apoptosis and mitophagy.

Patrice PETIT [1], Nathan Earl RAiNEY [1, 2], Ana SARIC [1, 3], François GONZALVEZ [1, 4]
[1] INSERM 1124, University Paris-Descartes, 45 rue des Saints-Pères, 75006 Paris, France
[2]CNRS UMR 7244, University Paris 13 Sorbonne Paris Cité, 93017 Bobigny, France
[3]Division of Molecular Medicine,Ruder Boškovic´ Institute, Zagreb, Croatia
[4]Ariad Pharmaceutical, Boston, USA
patrice.petit@inserm.fr

The Barth syndrome (BTHS) is caused by an inborn error of metabolism that manifests particular phenotypic features including altered mitochondrial membrane phospholipids (precisely cardiolipin non matiration), lactic acidosis, organic aciduria, skeletal muscle weakness and cardiomyopathy. The underlying cause of BTHS has been definitively traced to mutations in the tafazzin (TAZ) gene locus on chromosome X.

TAZ encodes a phospholipid transacylase that promotes cardiolipin acyl chain remodeling. Altered of tafazzin activity results in cardiolipin molecular species heterogeneity (with more satured spevies), increased levels of monolysocardiolipin and lower cardiolipin abundance. In skeletal muscle and cardiac tissue mitochondria these alterations in cardiolipin perturb compromise electron transport chain function and aerobic respiration. Decreased electron flow from fuel metabolism via NADH ubiquinone oxidoreductase activity leads to a buildup of NADH in the matrix space and product inhibition of key TCA cycle enzymes. As TCA cycle activity slows pyruvate generated by glycolysis is diverted to lactic acid. In turn, Cori cycle activity increases to supply muscle with glucose for continued ATP production. Acetyl CoA that is unable to enter the TCA cycle is diverted to organic acid waste products excreted in urine. Overall, reduced ATP production efficiency in BTHS is exacerbated under conditions of increased energy demand. Sarcomere assembly and myocardial contraction abnormalities occurred in the context of normal whole-cell ATP levels. Excess levels of reactive oxygen species mechanistically linked TAZ mutation to impaired cardiomyocyte function.

In an in vitro model of Barth syndrome, we demonstrated that the mitochondrially amplified apoptotic signal is fully inhibited due to the incapacity of caspase-8 to be fully activated and to recruit Bid. We also see that reactive oxygen species are produced in increased amount. The abnormal and deleterious mitochondria are also not susceptible to undergo mitophagy and then accumulates.

Prolonged deficiency in ATP production capacity and mitophagy inhibition underlies cell and tissue pathology that ultimately is manifest as dilated cardiomyopathy.

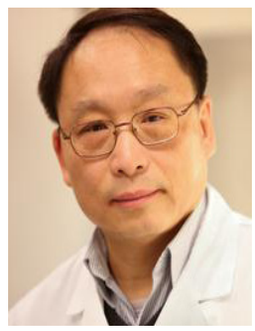
IL-20
Mitochondrial quality control and disease

Massimo Zeviani, Erika Fernandez-Vizarra, Alba Signes, Emanuela Bottani, Raffaele Cerutti, Ian Fearnley, Alan Robinson, Aurelio Reyes, Carlo Viscomi
Mitochondrial Biology Unit, MRC and University of Cambridge Cambrid , UK
mdz21@mrc-mbu.cam.ac.uk

The biogenesis and maintenance of mitochondrial electron transport chain (ETC) is an intricate process, associated with an expanding number of mitochondrial disorders. In addition to numerous chaperones participating in the formation of individual ETC complexes, the genetic characterization of specific human diseases has allowed the identification of factors that control the structural and functional integrity of the complexes in normal or stress-induced conditions. For instance, mutations in TTC19 have been associated with severe neurological phenotypes and mitochondrial respiratory chain complex III deficiency. Using a Ttc19-/- mouse model and TTC19 mutant cells from patients, we demonstrate that TTC19 binds to the fully assembled complex III dimer, i.e. after the incorporation of the iron-sulfur Rieske protein (UQCRFS1). The in-situ maturation of UQCRFS1 produces N-terminal polypeptides, which remain bound to holocomplex III. We show that, in normal conditions, these UQCRFS1 fragments are rapidly removed, but when TTC19 is absent they accumulate within complex III, causing its structural and functional impairment. Overall, these data indicate a role for TTC19 in a novel post-assembly quality control process, aimed at removing the mitochondrial targeting sequence of the UQCRFS1 precursor. A second example are mutations in APOPT1, associated with cavitating leukoencephalopathy and isolated mitochondrial cytochrome c oxidase (COX) deficiency. An Apopt1 knockout mouse model, created by using CRISPR-Cas9 technology in mouse embryos, recapitulates the biochemical hallmarks found in human patients. Although the molecular mechanisms underlying the biochemical defect remain elusive, we demonstrate that APOPT1 is rapidly degraded in standard conditions, but becomes stabilized during oxidative stress. APOPT1 mutant cell lines display reduced post-translational stability of MT-CO1 protein, a phenomenon which is complemented by re-expression of wild-type GFP-tagged APOPT1. These preliminary results suggest a role for APOPT1 in protecting COX from oxidative damage.

IL-21

Mechanisms regulating mitochondrial transport in neuronal degeneration and regeneration



Zu-Hang Sheng, Ph.D.
Senior Investigator, NINDS, NIH
Shengz@ninds.nih.gov

Mitochondria are cellular energy power plants essential for neuronal growth and function. Stationary mitochondria serve as local energy sources that constantly supply ATP. Neurons utilize specialized mechanisms driving mitochondria transport to and anchoring them in axons and at synaptic terminals. We previously identified syntaphilin as a static anchor specific for axonal mitochondria (Keng et al., Cell 2008). Deleting syntaphilin in mice dramatically increases axonal mitochondrial motility both in vitro and in vivo, thus providing a unique model for investigations into mechanisms regulating mitochondrial motility. In the presentation, I will discuss new mechanisms removing damaged mitochondria from distal axons in healthy and diseased neurons (Lin et al., Neuron 2017). Chronic mitochondrial stress is a central problem associated with neurodegenerative diseases. Early removal of defective mitochondria from axons constitutes a critical step of mitochondrial quality control. We investigate axonal mitochondrial response to mild stress in wild-type neurons and chronic mitochondrial defects in Amytrophic Lateral Sclerosis (ALS)- and Alzheimer's disease (AD)-linked neurons. We show that stressed mitochondria are removed from axons triggered by the bulk release of mitochondrial anchoring protein syntaphilin via a new class of mitochondria-derived cargos independent of Parkin, Drp1 and autophagy. Immuno-electron microscopy and super-resolution imaging show the budding of syntaphilin cargos, which then share a ride on late endosomes for transport toward the soma. Releasing syntaphilin is also activated in the early pathological stages of ALS- and AD-linked mutant neurons. Our study provides new mechanistic insights into the maintenance of axonal mitochondrial quality through SNPH-mediated coordination of mitochondrial stress and motility before activation of Parkin-mediated mitophagy. (Supported by the Intramural Research Program of NINDS, NIH)

IL-22
Proteolytic Control of Mitochondrial Function

Thomas Langer
Institute for Genetics and CECAD Research Center, University of Cologne, Cologne, Germany
office-langer@uni-koeln.de

Mitochondria are emerging as cellular signaling platforms deeply integrated into various cell survival and cell death cascades. Proteolytic events at the inner membrane (IM) represent central regulatory steps in these processes, emphasizing the importance of mitochondrial IM proteases beyond their roles as gatekeepers of protein quality. The m-AAA protease degrades EMRE in the IM, an essential subunit of the mitochondrial Ca2+ uniporter MCU. This ensures the assembly of gatekeeper subunits with MCU and prevents Ca2+ overload, opening of the membrane permeability transition pore and neuronal death. The IM proteases YME1L and OMA1 mediate the processing of the dynamin-like GTPase OPA1, balancing fusion and fission of mitochondrial membranes. YME1L also catalyzes the regulatory turnover of PRELID1, a lipid transfer protein in the intermembrane space that is required at an early stage in the synthesis of the mitochondrial phospholipid cardiolipin. OMA1 is a stress-activated peptidase, which ensures protein quality control, fine-tunes mitochondrial bioenergetic function and controls cellular apoptotic resistance. The rhomboid protease PARL cleaves the PTEN-induced kinase PINK1 in the IM, which regulates the activity of respiratory complex I and the clearance of damaged mitochondria by mitophagy. Moreover, PARL processes Smac/DIABLO allowing its release from mitochondria in apoptotic cells and ensuring the progression of apoptosis. IM proteases thus have a pivotal role in determining the form and function of mitochondria as well as the regulation of cell signaling and survival.