ISF Workshop

MITOCHONDRIA PAST & PRESENT:
Evolution, Proteostasis, Dynamics and Disease

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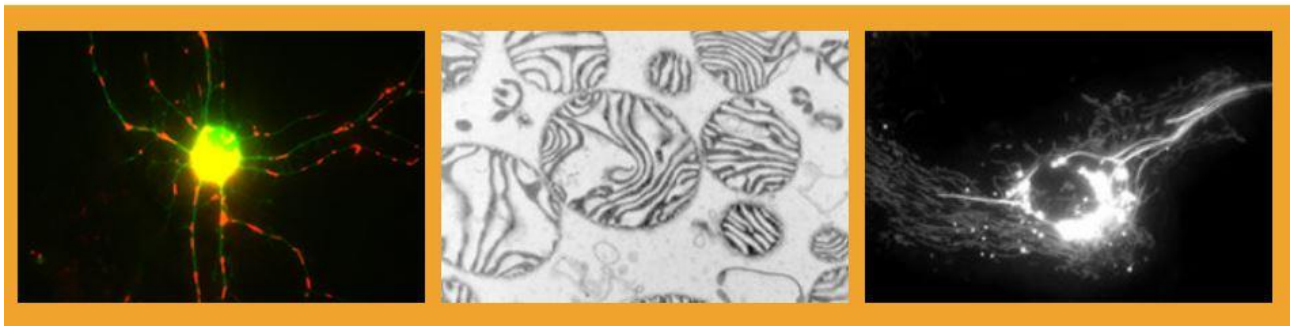

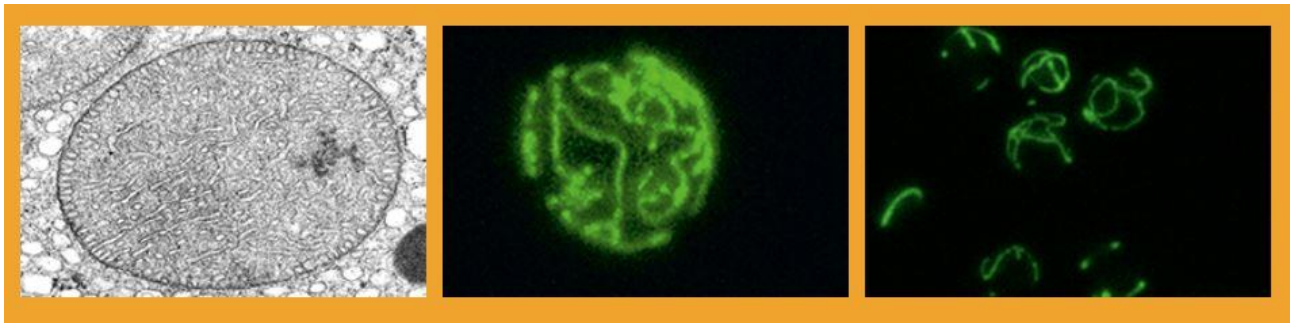

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Abstracts of Oral Presentations

Pre-Meeting Session

Pre-meeting session (Sunday, November 13, 2022 09:00)

Regulation of mitochondrial fission by changes in nutrient availability

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Fasting triggers diverse cellular and metabolic adaptations to facilitate organismal survival. During nutrient deprivation, increases in circulating fatty acids support mitochondrial respiration. The mechanisms driving mitochondrial adaptations and respiratory sufficiency during nutrient deprivation remain incompletely understood. We have found that extended fasting paradoxically stimulates the nutrient sensitive kinase mTORC2. This mTORC2 reactivation supports fasting-induced increases in mitochondrial fission and respiration in vivo. Accordingly, inactivation of mTORC2 in liver by knocking-out its critical regulator protein, Rictor (*Rictor*^{KO}), impairs fasting-induced increases in mitochondrial fission and dampens mitochondrial respiration. As a consequence, fasted *Rictor*^{KO} livers exhibit marked accumulation of liver triglycerides due to the failure to mobilize these lipid species. Using quantitative phosphoproteomics, we identified a new role for mTORC2 and its novel downstream targets in regulating mitochondrial fission via recruitment of regulatory proteins to mitochondrial associated membranes (MAMs), which are sites for ER-mediated mitochondrial fission. During nutrient surplus, mTOR complexes perform anabolic functions; however, paradoxical reactivation of mTORC2 during fasting plays an unexpected role in mitochondrial division and respiration.

Pre-meeting session (Sunday, November 13, 2022 09:00)

Mitochondrial morphology controls fatty acid utilization by regulating CPT1 function

Jennifer Ngo[1], Dong Wook Choi[2,3], Illana Stanley[2], Linsey Stiles[1], Anthony Molina[4], Pei-Hsuan Chen[2], Ana Lako[2], Chiao Han Sung[2], Nathanial Miller[1,5], Siyouneh Baghdasarian[1], Anthony Jones[1], Brett Roach[1], Doyeon Vasquez-Kim[1], Vincent Gutierrez[1], Karel Erion[1], Ajit Divakaruni[1], Marc Liesa[1,6], Nika Danial[2], Orian Shirihai[1]
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Changes in mitochondrial morphology are associated with nutrient utilization, but the precise causalities and the underlying mechanisms remain unknown. Using cellular models representing a wide spectrum of mitochondrial shapes, we show a strong linear correlation between mitochondrial fragmentation and

increased fatty acid oxidation (FAO) rates. Forced mitochondrial elongation following MFN2 over-expression or DRP1 depletion diminishes FAO, while forced elongation upon knockdown/knockout of MFN2 augments FAO as evident from respirometry and metabolic tracing studies. Importantly, genetic induction of fragmentation phenocopies the distinct cell type-specific biologic functions of FAO. These include stimulation of gluconeogenesis in hepatocytes, induction of insulin secretion in islet β-cells exposed to fatty acids, and survival of FAO-dependent lymphoma subtypes. We find that fragmentation stimulates long-chain but not short-chain FAO, identifying CPT1 as the downstream effector of mitochondrial morphology in regulation of FAO. Mechanistically, we determined that fragmentation reduces malonyl-CoA inhibition of CPT1, while elongation increases CPT1 sensitivity to malonyl-CoA inhibition. Overall, these findings underscore a physiologic role for fragmentation as a mechanism whereby cellular fuel preference and FAO capacity are determined.

Pre-meeting session (Sunday, November 13, 2022 09:00)

Stopping thieving mitochondrial thioesterases: the consequences of not stealing from beta-oxidation

Erin Seifert
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Acyl-CoA thioesterases (Acot) in the mitochondrial matrix hydrolyze acyl-CoAs (ACoA) of various chain lengths and so are well positioned to siphon off ACoAs from pathways that use them, such as β-oxidation. We previously overexpressed Acot2 (specific for long-chain fatty acyl-CoAs) in the liver of mice and found that β-oxidation was amplified, likely by an uncoupling effect caused by the fatty acids liberated by the Acot2 reaction (Moffat, 2014 PMID 25114170). To determine if endogenous Acot2 has a similar role in cells where it's highly expressed, Acot2 was depleted (Kd) from mouse striated muscle. And, to more broadly understand what Acot2 does and to attempt to define "mitochondrial lipid overload", we set up a matrix of conditions to vary the amount of lipid delivery and oxidation in the mice, and measured, in muscle, all β-oxidation intermediates, from C16:0-CoA to C4:0-CoA, then used these to model beta-oxidation "pseudo-flux" in all lipid abundance+flux conditions. Mitochondrial lipid overload in muscle in vivo was evident in the high-fat+low-flux condition regardless of Acot2 presence, and in low-fat+low-flux when Acot2 was absent, and was relieved when beta-oxidation flux accelerated. These scenarios were essentially apparent when we measured bioenergetics in isolated mitochondria, (i.e., under real flux conditions but entirely ex vivo), which further showed that Acot2-mediated fatty acid cycling was unlikely in muscle. Mitochondrial lipid overload was also observed in the high-fat+low-flux condition, but only when Acot2 was present. Without Acot2, beta-oxidation pseudo-flux in high-fat+low-flux condition resembled the low-fat+low-flux condition in Acot2-replete muscle, i.e., well-correlated, supporting no overload. The high-fat condition correlated with improved glucose oxidation in mice and less ceramide-derivative lipid in muscle. Thus, absence of Acot2 ACoA siphoning lead to a metabolic switch away from lipid uptake and oxidation in muscle when lipid supply was high, presumably avoiding mitochondrial lipid overload in vivo. Funding: NIH R01DK109100.

MYC regulates metabolic reprogramming and reliance on oxidative mitochondrial metabolism induced by androgen receptor inhibition in prostate cancer

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Advanced prostate cancers are treated with therapies targeting the androgen receptor (AR) signaling pathway. While many advanced tumors initially respond to AR inhibition, nearly all develop resistance. It is critical to understand how prostate tumor cells survive AR inhibition in order to exploit therapy-induced phenotypes, prior to the outgrowth of treatment-resistant disease. We interrogated clinical datasets, prostate cancer cell lines and patient-derived xenograft tissues using a range of approaches including transcriptional profiling, metabolic profiling, respirometry and western blotting to define metabolic reprogramming as a result of AR blockade. Transcriptional and metabolic profiling reveal widespread reprogramming of metabolism in prostate cancer cells that survive AR blockade. AR inhibition reduces glycolysis and increases reliance on mitochondrial oxidative metabolism. Robust mitochondrial elongation via DRP1 downregulation is observed in cells that survive AR inhibition. We define reduced MYC as a mediator of AR blockade-induced metabolic phenotypes, as restoring MYC increases glycolytic enzyme expression, restores DRP1-mediated mitochondrial fission, and reduces sensitivity to complex I inhibition in prostate cancer cells that survive antiandrogen treatment. In summary, our data suggest that AR blockade reprograms prostate cancer metabolism and increases dependence on oxidative mitochondrial metabolism through reduced MYC. Our results highlight the importance of defining regulators of treatment-induced metabolic reprogramming and treatment-associated vulnerabilities prior to tumor relapse.

Pre-meeting session (Sunday, November 13, 2022 09:00)

Mitochondrial bioenergetics and aging: Systemic mediators of physical and cognitive decline

Anthony Molina
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Progressive, systemic, mitochondrial bioenergetic decline is a hallmark of the aging process and has been linked to multiple age-related conditions, including physical and cognitive decline. Importantly, it is broadly recognized that the rate of bioenergetic decline with advancing age is highly heterogeneous, and is influenced by multiple factors; such as lifestyle, diet, exercise, and environmental exposures; that have cumulative effects over time. This presentation will present recent and ongoing studies from our team that are examining how behavioral factors such as nutrition, and intrinsic factors such as sex, influence trajectories of bioenergetic decline among older adults. This work is paving the way for the discovery of mito-active molecules, that are driving bioenergetic changes associated with human aging and disease progression.

Pre-meeting session (Sunday, November 13, 2022 11:00)

Immuno-metabolic dysregulation in macrophages during aging: Aging at the cross roads of inflammation and metabolism

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Microbiology, Immunology, and Molecular Genetics, UCLA, Los Angeles, CA, USA

Senescent cells are damaged or stressed cells that have undergone irreversible cell cycle arrest and secrete inflammatory cytokines and other factors, known as the senescence associated secretory phenotype, SASP. In a recent manuscript we showed that senescent cells progressively accumulate in aging metabolic tissues, leading to activation of macrophages via inflammatory cytokines found in the SASP, resulting in degradation of NAD+ via the NAD+ hydrolase enzyme CD38. A major barrier to better understanding how senescence cells drive inflammaging and altered tissue NAD+ metabolism is identifying the cell types that undergo senescence during the aging process. This is due to the fact that detecting senescent cells in vivo is very difficult due to limited techniques and biomarkers to identify them at the single cell level. To answer this question, we utilized next generation single-cell RNA-sequencing of the liver tissue from old and young mice. We revealed that a large fraction of old Kupffer cells (24+ months), resident macrophages of the liver, and not other cell types have increased expression of cellular senescence associated genes and inflammatory cytokines. These data suggest that a subset of macrophages undergo senescence in aging tissues and contribute to aging-related diseases. We hypothesize that macrophages are a key source of senescent cells during the aging process and are a key driver of inflammation, altered NAD+ metabolism, and immune cell dysfunction in aging. To test this hypothesis, we have developed an in vitro senescent macrophage system that we will use to characterize the unique biology of senescent macrophages to better identify and target them for therapeutic purposes, investigate their link to altered NAD+ metabolism observed in aging, and to determine what role they play in aging using in vivo studies.

Pre-meeting session (Sunday, November 13, 2022 11:00)

Mitochondrial calcium spoils the romance between lipid droplets and mitochondria

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Our group has recently reported that BAT harbor two separate subsets of mitochondria: (1) peridroplet mitochondria (PDM) which are physically attached to lipid droplets (LD) and which contribute to the formation of LD; (2) cytosolic mitochondria (CM) which are separated from the LD and contribute most to uncoupled respiration. These two sets of population display different proteomic profiles that explain their different metabolic features. Cold-adaptive thermogenesis induce dynamic changes in these mitochondrial pools leading to a decrease in PDM population. A sharp increase in mitochondrial Ca2+ marks the activation of the brown adipose tissue (BAT) thermogenesis, yet the mechanisms preventing Ca2+ deleterious effects are poorly understood. We have shown that adrenergic stimulation of BAT activates a PKA-dependent mitochondrial Ca2+ extrusion via the mitochondrial Na+/Ca2+ exchanger, NCLX. Adrenergic stimulation of NCLX-ablated brown adipocytes (BA) induces a profound mitochondrial Ca2+ overload and impaired uncoupled respiration. Our preliminary results suggest that CM and PDM populations also differ in their response to Ca2+ signaling, in their preponderance to mPTP opening and in its metabolic adaptation to cold exposure, with ultimate affect lipid mobilization and adaptation upon adrenergic stimulation.

Pre-meeting session (continued) (Sunday, November 13, 2022 11:00)

Sex- and tissue-dependent determinants of mitochondrial activity in sedentary and exercised states

Laurent Vergnes[1], Anvi Brahmbhatt[1], Maggie Polite[1], **Karen Reue**[1]
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Mitochondrial function in metabolic tissues including adipose tissue and skeletal muscle influences cardiovascular health. Biological sex influences mitochondrial number and/or activity and adaptations that occur in response to chronic exercise. The role of specific sex components (gonadal sex vs. chromosomal sex), and the changes occurring across adipose and muscle tissue types in response to exercise, have not been characterized. We determined metabolic and mitochondrial parameters in adipose tissues (gonadal and inguinal white adipose, and interscapular brown adipose) and multiple skeletal muscle types (quadriceps, soleus, gastrocnemius, tibialis, and extensor digitorum longus) from Four Core Genotypes mice before and after chronic exercise. This mouse model allows the identification of effects of gonadal sex (ovaries vs. testes) and of chromosomal sex (XX vs. XY chromosomes). We identified distinct effects of chromosomal and gonadal sex on mitochondrial DNA content (mtDNA) and respiratory activity that varied with muscle and adipose tissue type. Chronic exercise altered tissue mass, glucose levels, grip strength, mtDNA, and respiratory activity in most adipose and muscle types in a sex-dependent manner, with interactions between exercise and either gonadal sex or chromosomal sex. These findings demonstrate that intrinsic sex differences in mitochondrial parameters in key metabolic tissues likely contribute to sex differences in muscle and adipose tissue function and adaptation to exercise.

Mitochondrial uncouplers induce proton leak by activating AAC and UCP1

Ambre Marguerite Solange Bertholet
Physiology, UCLA, Los Angeles, California, USA

Mitochondria generate heat due to H^+ leak (I_H) across their inner membrane. I_H results from the action of long-chain fatty acids (FA) on uncoupling protein 1 (UCP1) in brown fat and ADP/ATP carrier (AAC) in other tissues, but the underlying mechanism is poorly understood. Because no pharmacological activators of I_H via UCP1 and AAC are known, I_H is induced by protonophores such as 2,4-dinitrophenol (DNP) and cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP) that are thought to increase I_H independently of membrane proteins. Protonophores are highly effective in combating obesity, insulin resistance, type II diabetes, and fatty liver in animal models, but because they indiscriminately increase H^+ conductance across all biological membranes and have adverse side effects, their use in humans is restricted. Here we report the first direct measurement of I_H induced by common protonophores and discover that their mechanism of action in mitochondria was significantly misunderstood. I_H induced by DNP and other protonophores depends on AAC and UCP1[1]. Using molecular structures of AAC, we perform a computational analysis to determine the binding sites for protonophores and long-chain FA and find that they overlap with the putative ADP/ATP binding site. We also develop a mathematical model that proposes a mechanism of uncoupler-dependent I_H via AAC. Thus, common protonophoric uncouplers emerge as the first synthetic activators of I_H via UCP1 and AAC identified to date, paving the way for the development of new and more specific activators of these two proteins.

[1]Bertholet AM, Natale AM, Bisignano P, Suzuki J, Fedorenko A, Hamilton J, Brustovetsky T, Kazak L, Garrity R, Chouchani ET, Brustovetsky N, Grabe M, Kirichok Y. Nature. 2022 Jun;606(7912):180-187.

Mitochondrial architecture and membranes (Sunday, November 13, 2022 14:00)

Keynote address:
Uncovering the function and mechanisms of mitochondrial derived vesicles.

Heidi McBride
Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada

Mitochondria are central hubs of cellular metabolism and tightly connected to signaling pathways. The dynamic plasticity of mitochondria to fuse, divide and contact other organelles to flux metabolites is central to their function. To ensure bona fide functionality and signaling-interconnectivity, diverse molecular mechanisms evolved. An ancient mechanism is the generation of mitochondrial-derived vesicles (MDVs) that shuttle selected mitochondrial cargoes to target organelles. Just recently, we gained significant insight in the mechanisms and functions of MDV transport ranging from their role in mitochondrial quality control to immune signaling, thus demonstrating unexpected and diverse physiological aspects of MDV transport. I will highlight the origin of MDVs, their biogenesis and their cargo selection, with a specific focus on the contribution of MDV transport to signaling and cell death pathways. I will present unpublished work that provides insights into the roles of MDVs in Parkinsons disease pathways.

Mitochondrial architecture and membranes (Sunday, November 13, 2022 14:00)

Mapping mitochondrial contact sites with diverse cellular organelles

Maya Schuldiner
Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel

To optimize their multiple cellular functions, mitochondria must collaborate and communicate with surrounding organelles. A common way of communication between organelles is thorough physical membrane contact sites where membranes of two organelles are tethered, facilitating close-range interactions. These are important for exchange of small molecules, intracellular signaling, inheritance and division. To uncover the extent of mitochondrial contact sites and their physiological roles we created a split fluorescence reporter for yeast mitochondrial contact sites by which one part of a fluorophore is fused to the outer membrane of mitochondria while the other is fused to the outer surface of the membrane of all other organelles. We extensively validated that these report on *bona fide* contact sites between mitochondria and all other organelles such as lipid droplets, peroxisomes and the nucleus. I will discuss how these are now being used in high content screens to uncover novel tethers and regulatory molecules as well as the physiological roles of the contacts.

Mitochondrial architecture and membranes (Sunday, November 13, 2022 14:00)

ER-mitochondrial contact dynamics

Gyorgy Hajnoczky
MitoCare, Pathology & Cell Biology, Thomas Jefferson University, Philadelphia, Pa, USA

Local communication between intracellular organelles at membrane contacts is a burgeoning topic in cell biology and medicine. Endoplasmic Reticulum-Mitochondria contacts (ERMCs) support many functions including metabolism, neuronal and immune activities, and their impairment causes disorders across organs. ERMC structure and function changes with the state of the cells, but how ERMCs are formed and dissolved is yet to be determined. In this study of ERMCs structure and function we employed and advance in the synthetic linker strategy to study the role of a particular, important physiological tethering species, the IP3R. With this technique we were able to show that IP3Rs constantly move in and out of ERMCs and that trapping them at the ERMCs instantaneously improves the calcium signal propagation from the ER to the mitochondria leading to enhanced activation of energy metabolism. We expect a similar strategy could underpin the investigation of the dynamics of other natural tethers and interorganellar contacts as well.

Mitochondrial architecture and membranes (Sunday, November 13, 2022 14:00)

Mitochondrial Derived Vesicles (MDVs) retain membrane potential and contain a functional ATP synthase

Reut Noa Hazan[1], Dvora Lintzer[1], Tamar Ziv[2], Koyeli Das[1], Irit Rosenhek-Goldian[3], Ziv Porat[4], Hila Ben Ami Pilo[5], Sharon Karniely[6], Ann Saada[7], Neta Regev-Rudzki[5], Ophry Pines[1]
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Vesicular transport is a means of communication. While cells can communicate between each other via secretion of extracellular vesicles, less is known regarding organelle-to organelle communication, in particularly in the case of mitochondria. Mitochondria are responsible for the production of energy and for essential metabolic pathways in the cell, as well as fundamental processes such as apoptosis and aging. Here we show that functional mitochondria, isolated from Saccharomyces cerevisiae release vesicles, independent of the fission machinery. We were then able for the first time to isolate these Mitochondrial Derived Vesicles (MDVs) and found that they are relatively uniform in size, of about 100nm and carry selective protein cargo including enrichment of ATP synthase subunits. Remarkably, we further found that these MDVs harbor a functional ATP synthase complex. Moreover, we demonstrate that these vesicles have a membrane potential, produce ATP, and seem to fuse with naive mitochondria. Our findings reveal a possible delivery mechanism of ATP producing vesicles, which can potentially regenerate ATP deficient mitochondria and may participate in organelle to organelle communication.

Mitochondrial architecture and membranes (Sunday, November 13, 2022 14:00)

Intramitochondrial dynamics of cristae and crista junctions

Andreas Reichert

Institute of Biochemistry and Molecular Biology I, Heinrich-Heine-University Duesseldorf, Düsseldorf, Germany

Since the first papers by Hackenbrock more than 50 years ago mitochondria are known to exist in a ‘condensed’ or an ‘orthodox’ state depending on their bioenergetic status. The notion that the inner mitochondrial membrane (IMM) can reshape under different physiological conditions or during apoptosis is supported by many later studies. Altered IMM ultrastructure is well known to be implicated in numerous neurological and muscular disorders. We showed recently using state-of-the-art live-cell stimulated emission depletion (STED) super-resolution nanoscopy that cristae as well as crista junctions (CJs) are dynamically fusing and dividing in a reversible and balanced manner at a timescale of seconds. Moreover, several lines of evidence strongly suggest the formation of transient cristae vesicles with dynamic changes of the membrane potential. These dynamics processes depend on the MICOS complex, known to be required for formation of crista junctions and contact sites. We showed that loss of Mic13, a subunit of the MICOS complex which is causally linked to mitochondrial encephalopathy with liver dysfunction in humans, impairs cristae dynamics. Here I will discuss how this novel concept of transient formation of cristae vesicles and cristae dynamics could shape mitochondrial ultrastructure and why this has fundamental implications for the generation of ATP via oxidative phosphorylation and other mitochondrial functions.

Protein and RNA targeting to mitochondria (Sunday, November 13, 2022 16:15)

Mitochondrial protein biogenesis: A big challenge for eukaryotic cells

Johannes Herrmann

Cell Biology, University of Kaiserslautern, Kaiserslautern, Germany

Most mitochondrial proteins are synthesized as cytosolic precursor proteins before they are imported into mitochondria. The molecular details of the mitochondrial import reactions were dissected in great mechanistic detail by the use of very powerful biochemical methods in vitro. However, recent in vivo studies by us and others showed that mitochondrial precursor proteins have a surprisingly complex biology before they reach the mitochondrial surface. We realized that mitochondrial precursor proteins explore the cytosol, get in contact with components of the proteasome and chaperone network and often end up on the surface or even the lumen of other cellular compartments. Under conditions of reduced mitochondrial protein uptake, these cytosolic precursor proteins pose a severe threat to cytosolic proteostasis. And vice versa, improving mitochondrial protein import can suppress the proteotoxic effect of aggregation-prone proteins in the cytosol. Thus, mitochondrial precursors are central constituents of the cytosolic proteostasis network and to deal with these proteins is a big challenge for eukaryotic cells. In my talk, I will give an overview about our current knowledge of these early reactions in mitochondrial protein targeting and present recent data from our team.

Protein and RNA targeting to mitochondria (Sunday, November 13, 2022 16:15)

New insights into the biogenesis of mitochondrial outer membrane proteins

Layla Drwesh, Jialin Zhou , **Doron Rapaport**
Interfaculty Institute of Biochemistry, University of Tübingen, Tübingen, Germany

Mitochondrial outer membrane proteins are encoded by nuclear DNA, translated on cytosolic ribosomes, and are then targeted to the organelle and inserted into its OM by import factors. Using various model signal-anchored (SA) proteins and a broad set of assays, we reconstituted the early steps of their biogenesis. We identified a subset of molecular (co)chaperones that interact with newly synthesized SA proteins, namely, Hsp70 and Hsp90 chaperones and co-chaperones from the Hsp40 family like Ydj1 and Sis1. These interactions were mediated by the hydrophobic transmembrane segments of the SA proteins. We could further demonstrate direct interaction of peptides corresponding to the transmembrane segments of SA proteins with the (co)chaperones and reconstitute *in vitro* the transfer of such peptides from the Hsp70 chaperone to the mitochondrial Tom70 receptor.

To obtain new insights on the biogenesis of multi-span OM proteins, we utilized yeast mitochondria and the multi-span protein Om14. Testing different truncation variants, we show that while only the full-length protein contains all the information that assure perfect targeting specificity, shorter variants are targeted to mitochondria with compromised fidelity. We further demonstrate that Mim1 and Porin(VDAC) support optimal membrane integration of Om14 but none of them is absolutely required. Collectively, our findings suggest that MOM multi-span proteins follow different biogenesis pathways in which proteinaceous elements and membrane behavior contribute to a variable extent to the combined efficiency.

Protein and RNA targeting to mitochondria (Sunday, November 13, 2022 16:15)

Pathways and machineries that mediate and regulate mitochondrial protein trafficking

Toshiya Endo
Faculty of Life Sciences, Kyoto Sangyo University, Kyoto, Japan

Mitochondria are central to energy production, metabolism and signaling, and apoptosis. To make new mitochondria from preexisting mitochondria, the cell needs to import mitochondrial proteins from the cytosol into the mitochondria with the aid of translocators in the mitochondrial membranes. Thanks to the development of cryo-EM technology, high-resolution structures of mitochondrial translocators including the TOM and SAM complexes in the outer membrane have become available, which opens up a new era of studies on the structures, functions, and dynamics of the mitochondrial import system. In addition, it has become evident that protein targeting tends to be more error-prone than previously envisaged, and cells are thus equipped with a system to ensure precise subcellular localization of newly synthesized proteins through not only degradation of mistargeted proteins but also “iterative re-routing” of protein delivery. This system likely requires energy to operate, so it includes ATPases like Msp1 on mitochondria and Spf1 on the ER in yeast cells. Here I will introduce and discuss recent results from our lab on these topics.

Export of mitochondrial double-stranded RNA from mitochondria

Carla Koehler[1], Matthew Krieger[1], Melania Abrahamian[1], Sean Atamdede[1], Connor Short[1], David Shackelford[1], Xia-Wu Gai[2], Michael Teitell[1]
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The mitochondrial inner and outer membranes serve as barriers with transporters to regulate the movement of macromolecules. In addition to proteins and mitochondrial DNA, a diverse cohort of RNA species including mitochondrial double stranded RNAs (mtdsRNAs), cytosolic tRNAs, small and long non-coding RNAs, and viral RNAs, are imported as well as exported from mitochondria. However, the specific channels for RNA transport have not been demonstrated and the translocation route may vary by species. Here we begin to characterize candidates that participate in an export pathway for mtdsRNAs from the mitochondrial matrix to the cytosol in cultured cells. Downregulation of SUV3 resulted in accumulation of mtdsRNAs in the matrix, whereas downregulation of PNPase resulted in export of mtdsRNAs to the cytosol. Inhibiting or downregulating outer membrane proteins VDAC and BAK/BAX or inner membrane proteins PHB1/2 blocked the export of mtdsRNAs to the cytosol. The cytosolic mtdsRNAs subsequently localized to large granules that also contained the stress protein TIA1 and activated the Type-1 Interferon pathway. Abundant mtdsRNAs were detected in a subset of Non-Small Cell Lung Cancer cell lines, indicating relevance in cancer biology. In sum, we propose that mtdsRNA is a damage-associated molecular pattern that is exported in a regulated manner under conditions of mitochondrial stress.

Specialized ribosomes in the translation of mitochondrial and peroxisomal proteins

Jeffrey Gerst, Raman Singh
Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel

Genome duplication in eukaryotes created paralog pairs of genes encoding ribosomal proteins (RPs) with extremely high sequence similarity/identity. In the yeast, S. cerevisiae, 3/4 of the RP genes underwent duplication, which allows for the creation of over 10^{17} possible different ribosomes. Yet yeast possess only 2-3x10^5 ribosomes and thus questions arise regarding their actual composition in cells, given that these RP paralogs are co-expressed. In theory, substitutions in the RP composition of ribosomes could allow for the specialized translation of different subsets of mRNAs, however, the evidence for specialized ribosomes and paralog-specific translational control is limited.

Initial studies by ourselves (Segev and Gerst, 2018 *J. Cell Biol.* and reviewed in Gerst 2018 *Trends Genet*) revealed that specific RP paralogs can confer the effective translation of nuclear-encoded mitochondrial messages, whereas their partner paralogs could not, despite being identical at the protein coding level. In our recent work, we have screened new yeast RP gene deletion libraries and phenotyped the individual strains. We found that deletions in one RP gene can phenocopy those of a different RP gene, allowing for hierarchal clustering of the phenotypes and predictive analysis of which paralogs are likely to act together within the same ribosome. This approach led us to define the composition of ribosomes required for growth on oleate and glycerol, which necessitate the efficient translation of peroxisomal and mitochondrial proteins, respectively. In parallel, studies into the translatomes of paralogs required for growth on oleate, along with mass spectometry analysis of both monosome and polysome composition of ribosomes containing these paralogs, as well as genetic studies, all point towards a novel mechanism by which specialized ribosomes are created via the recruitment of specific ribosome-associated proteins (RAPs). Ribosomes varying in RP and RAP composition confer specialized functions and, thus, RP paralog specificity defines a novel means of translational control.

Association and transport of mRNA with mitochondria in neurons

Yoav Arava

Biology, Technion, Israel Institute of Technology, Haifa, Israel

Neurons are cells with high energetic demands. As such, they are enriched in mitochondria. Furthermore, mitochondria are needed for neuronal growth and synaptic activity. Mitochondria contain hundreds of proteins, most of them derived from nucleus-transcribed mRNAs, and need to be transported to distant mitochondria. It is hypothesized that these mitochondria rely on local translation of nuclear-encoded mRNAs for their maintenance. Accordingly, mRNAs, ribosomes, and translation factors need to be transported to neuronal extremities to allow such local synthesis. The transport mechanisms of these elements are largely unknown. We propose that transport of these elements occurs through association with moving mitochondria.

Here we show by fractionation analysis that mRNAs of nuclear-encoded mitochondrial genes are associated with mitochondria, in both a neural-like cell line and motor neuron axons. Furthermore, by applying the MS2 mRNA visualization system we show that one of these mRNAs, Cox7c, is not only associated but also co-transported with mitochondria along neurites. Importantly, a translational process as well as the mitochondrial targeting sequence (MTS) are important for localization. These results reveal that mRNAs encoding mitochondrial proteins are associated and transported with mitochondria in a manner that might involve translation. We also identified mRNAs that do not encode mitochondria proteins to be associated with neuronal mitochondria; these are likely associated through elements outside the coding region. Localization analysis suggest that such elements are located within the 3`UTR. Altogether, our results suggest that mitochondria are associated with diverse types of mRNAs while in neuronal extensions. This suggest that mitochondria are self-reveal while moving along neurons and may serve as a shuttle to deliver mRNAs to distant sites.

Elucidating the cellular functions of Timm50, a mitochondrial transport protein, in neurons and astrocytes

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The human mitochondrial proteome consists of about 1400 different proteins, the majority of which are synthesized in the cytosol and subsequently transported into the various mitochondrial compartments. Several dedicated complexes mediate the transport of imported precursor proteins. We focus on the TIM23 complex, which mediates mitochondrial import of all matrix proteins as well as some of the inner membrane and inter membrane space (IMS) proteins. Timm50 is an essential component of the TIM23 complex and the first subunit that interacts with precursor proteins as they emerge into the IMS from the outer membrane. Subsequently, Timm50 hands over the precursor to the channel`s core, composed of the two essential proteins Timm23 and Timm17. Essentially, hundreds of proteins rely on proper function of Timm50 in order to reach their destination. Therefore, defects in Timm50 function are expected to lead to severe phenotypes, and indeed, Timm50 mutant forms were recently linked to an epileptic encephalopathy disease. Our goal is to study the neuronal functions of Timm50 in mouse primary neuronal cultures.

We applied knockdown techniques to manipulate the expression levels of Timm50 and examined the changes it caused to several key mitochondrial and neuronal features. Using cortical neuronal cultures, we show that Timm50 knockdown leads to specific destabilization of the TIM23 core import complex. This in turn causes a reduction in the levels of several oxidative phosphorylation complexes and has a deteriorating effect on mitochondrial shape and size, mitochondrial mobility along neurites, oxygen consumption and energy production. Additionally, we show that Timm50 knockdown neurons tend to be more electrically active.

Our approach allows us to study and manipulate Timm50 expression, for the first time in a controlled neurological system, in order to elucidate its neuronal roles and the molecular basis of its disease phenotypes.

The energetic cost of life doubles, and the rate of aging increases in fibroblasts with mitochondrial OxPhos Defects

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Mitochondrial respiratory chain (RC) dysfunction shortens lifespan in humans, but the underlying cellular mechanisms linking RC defects to lifespan remain unclear. To examine this question, we longitudinally tracked cellular aging trajectories in SURF1 mutant (n=3) and healthy fibroblasts (n=3) for up to 250 days, from early passages until replicative senescence. For up to 20 time points for each donor, we measured bioenergetic function (Seahorse), mtDNA integrity, secreted extracellular factors, telomere length, RNAseq, and genome-wide DNA methylation. Bioenergetically, SURF1 mutant cells exhibited an overall 85% higher total ATP production per unit of cell volume, reflecting a near doubling of energy consumption per unit of cell volume – or "hypermetabolism". This hypermetabolic phenotype was stable throughout the lifespan, indicating that sustaining life in SURF1 mutant cells, despite their significantly reduced division rate, is more energetically demanding. This hypermetabolic state was associated with mtDNA instability, activation of the integrated stress response (ISR), and an age-related secretory profile including GDF15 and extracellular cell-free mtDNA. Moreover, hypermetabolic cells exhibited accelerated telomere erosion per cell division, and a 53% lower Hayflick limit. We confirm the generalizability of these findings in healthy fibroblasts by pharmacologically inhibiting complex V (oligomycin, 1 nM), which increased cellular metabolic rate by 105%, accelerated telomere shortening and the onset of secreted aging markers, and reduced the Hayflick limit by 40%. Our combined longitudinal RNAseq & DNA methylation dataset in both cellular models highlight conserved pathways that may transduce RC dysfunction into hypermetabolism. This multi-omic dataset, which also includes other biochemical and endocrine perturbations, further reveals that energy consumption rate predicts the rate of molecular aging and magnitude of lifespan reduction. Thus, our longitudinal results across two models of RC dysfunction in patient-derived fibroblasts identify a cell-autonomous response that increases the energetic cost of living and recapitulates key molecular hallmarks of human aging.

Hiding in plain sight: the role of the mitochondrial fusion in shaping heteroplasmic mtDNA transmission across generations

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Deleterious and intact mitochondrial DNA (mtDNA) mutations frequently co-exist (heteroplasmy). Such mutations likely survive and are inherited due to complementation via the intracellular mitochondrial network. Hence, we hypothesized that compromised mitochondrial fusion would hamper such complementation, thereby affecting heteroplasmy inheritance. To test this hypothesis, we assessed heteroplasmic levels in three *Caenorhabditis elegans* strains carrying different heteroplasmic mtDNA deletions (ΔmtDNA) in the background of mutant mitofusin, *fzo-1*. Animals displayed severe embryonic lethality and developmental delay. Strikingly, these phenotypes were relieved during subsequent generations in association with complete ΔmtDNA removal. The rates of deletion loss negatively correlated with the size of mtDNA deletions, suggesting that mitochondrial fusion is essential and sensitive to the nature of the heteroplasmic mtDNA mutations. Introducing the ΔmtDNA into a *fzo-1;pdr-1*;+/ΔmtDNA (PARKIN ortholog) resulted in a skewed mendelian progeny distribution, in contrast to the normal distribution in the *fzo-1*;+/ΔmtDNA mutant and severely reduced brood size. Notably, the ΔmtDNA was lost across generations in association with improved phenotypes. Taken together, our findings show that when mitochondrial fusion is compromised, deleterious heteroplasmic mutations cannot evade natural selection while inherited through generations.

Evolution, inheritance and aging (Monday, November 14, 2022 09:00)

Mitochondrial transcription and mito-nuclear gene expression coordination diverge during metazoan evolution

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Mitochondrial DNA (mtDNA) transcription has been mainly studied in model organisms, and mostly in vitro. Previously we have adapted the precision run-on transcription sequencing (PRO-seq) to assess mtDNA sequencing in a variety of organisms. This study revealed differences in patterns of mtDNA transcription especially between drosophila, C. elegans and mammals. We noticed that changes in mtDNA gene order correlated with the emergence of several polycistrons per mtDNA strand in drosophila and heavy strand only mtDNA transcription in the worm. We thus studied mtDNA rearrangements across metazoan evolution, while analyzing whole mtDNA sequences from more than 8,000 different species. This analysis unearthed conservation of mtDNA gene order within most phyla, yet great differences between phyla. As similar to drosophila most arthropod species (65%) displayed an alternating-strand gene block organization we hypothesized that such conserved organization associate with a certain mtDNA transcriptional pattern. Gene-gene junction analysis using RNA-seq data from arthropods revealed that gene-gene junctions within the same strand had significantly higher sequencing reads count as compared to junctions between genes encoded by different strands. This was not observed in any available chordate. This suggested, that in different from chordates, gene blocks in the mtDNA of arthropods mark the beginning and ends of mtDNA polycistrons. Taken together, the selective constraints over mtDNA genomic organization within taxa, along with the impact of gene organizational changes on mtDNA transcription suggests that (A) there are previously overlooked regulatory elements throughout the mtDNA and (B) underlines the importance of investigating mitochondrial regulation across evolution, even using non-model organisms.

Evolution, inheritance and aging (Monday, November 14, 2022 09:00)

Solving the paradox of FH: the TCA pro-survival versus the tumor suppressor function

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Several tumor suppressor genes do not follow the canonical function of cell cycle repressors. For example, fumarate hydratase (FH) is an evolutionary conserved TCA cycle enzyme that reversibly catalyzes the hydration of Fumarate to L-malate and has a moonlight function in the DNA damage response (DDR). Interestingly, despite this enzyme`s essential role in central carbon metabolism, FH is knocked out or inactive in several tumors. Accordingly, FH has a contradictory cellular function, as it is pro-survival through its role in the TCA cycle, yet its loss can drive tumorigenesis, thus, supporting its role as a tumor suppressor. Here, we solved this contradiction by determining the molecular mechanisms that allow the cells to survive and even proliferate upon FH loss. We found that the cells` response to FH loss is separated into two distinct time frames based on cell proliferation and DNA damage repair. During the early stages of FH loss, the cells` proliferation and DNA damage repair are inhibited. However, over time the cells overcome the FH loss and form knockout clones, indistinguishable from WT cells in their proliferation rate. Due to the FH loss effect on DNA damage repair, we assumed that the recovered cells bear adaptive mutations. Therefore, we applied whole-exome sequencing to identify such mutated genes systematically. Indeed, we identified recurring mutations in genes belonging to the central oncogenic signaling pathways, such as JAK/STAT, which we validated to be impaired in FH-KO clones. Intriguingly, we demonstrated that these adaptive mutations are responsible for FH-KO cell proliferation under TCA cycle malfunction.

Evolution, inheritance and aging (Monday, November 14, 2022 09:00)

Age-related accumulation of de novo mitochondrial mutations in mammalian oocytes and somatic tissues

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Mutations create genetic variation for other evolutionary forces to operate on and cause numerous genetic diseases. Nevertheless, how de novo mutations arise remains poorly understood. Progress in the area is hindered by the fact that error rates of conventional sequencing technologies (1 in 100 or 1,000 base pairs) are several orders of magnitude higher than de novo mutation rates (1 in 10,000,000 or 100,000,000 base pairs per generation). Moreover, previous analyses of germline de novo mutations examined pedigrees (and not germ cells) and thus were likely affected by selection. Here, we applied highly

accurate duplex sequencing to detect low-frequency, de novo mutations in mitochondrial DNA (mtDNA) directly from oocytes and from somatic tissues (brain and muscle) of 36 mice from two independent pedigrees. We found mtDNA mutation frequencies 2- to 3-fold higher in 10-month-old than in 1-month-old mice, demonstrating mutation accumulation during the period of only 9 mo. Mutation frequencies and patterns differed between germline and somatic tissues and among mtDNA regions, suggestive of distinct mutagenesis mechanisms. Additionally, we discovered a more pronounced genetic drift of mitochondrial genetic variants in the germline of older versus younger mice, arguing for mtDNA turnover during oocyte meiotic arrest. Our study deciphered for the first time the intricacies of germline de novo mutagenesis using duplex sequencing directly in oocytes, which provided unprecedented resolution and minimized selection effects present in pedigree studies. Moreover, our work provides important information about the origins and accumulation of mutations with aging/maturation and has implications for delayed reproduction in modern human societies. Furthermore, the duplex sequencing method we optimized for single cells opens avenues for investigating low-frequency mutations in other studies.

Evolution, inheritance and aging (Monday, November 14, 2022 09:00)
Oral presentation

A billion-year trend of amino acid substitutions in the mitochondrial genome

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It has been shown that the rates of reciprocal amino acid substitutions in prokaryotic and eukaryotic organisms are not balanced, leading to the long-term increase (i.e. ‘gainers’) or decrease (i.e. ‘losers’) in the frequency of some amino acids. However, the evolutionary driving forces establishing this trend are still unknown. Here, focusing on the strongly asymmetrical mutational spectrum of the mitochondrial genome (an excess of G to A and T to C, light chain notation), we predicted the preferential direction of amino acid substitutions from losers (LeuTT, Phe, Cys, Trp, Gly, and Val) to gainers (Pro, His, Gln, Asn, Lys, and Thr). Analysing collections of nonsynonymous mtDNA mutations from human cancers (PCAWG), human pathogenic mutations (MitoMap database), human population polymorphisms, and mtDNA polymorphism from hundreds of vertebrate species we observed that the vast majority of substitutions are indeed in the expected direction: from losers to gainers. Moreover, the observed bias is the most pronounced in datasets where mutagenesis is stronger than selection (cancer and human pathogenic mutations for example). Comparing the amino acid composition of mtDNA genes between orthologs of mitochondrial genes in alpha-proteobacteria, fungi, plants, invertebrates, and five classes of vertebrates, we observed a global billion-year trend: losers become rarer while gainers become more frequent among these taxa. These results are in line with the accumulation of slightly-deleterious variants (i.e. from losers to gainers) in mtDNA from the moment of endosymbiosis emergence till the current days due to genetic drift, which becomes stronger from bacteria to vertebrates.

Programming cell fate by mitochondrial proteolysis

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Metabolic reprogramming of mitochondria occurs during development, cell differentiation, in ageing and disease and is coupled to changes in mitochondrial mass and shape. Mitochondrial proteases are emerging as central regulators of these adaptive responses. The -AAA protease YME1L regulates mitochondrial fusion via OPA1 in concert with the stress-activated peptidase OMA1 and couples mitochondrial shape and metabolic function. YME1L activation promotes growth of pancreatic ductal adenocarcinoma cells and preserves the self-renewal capacity of adult neural stem cells. OMA1 also promotes the integrated stress response by DELE1 cleavage and protects against cardiomyopathy induced by OXPHOS dysfunction. Proteolysis by the *m*-AAA protease, associated with various neurodegenerative diseases, broadly rewires the mitochondrial proteome in response to bioenergetic cues and ensures mitochondrial gene expression and OXPHOS-dependent cell growth. The function of the mitochondrial rhomboid protease PARL, an intramembrane cleaving serine peptidase, has been linked to the assembly of respiratory complex III, coenzyme Q synthesis, PINK1-Parkin dependent mitophagy and apoptosis. Recent findings on novel roles of these proteases for the functional plasticity of mitochondria and for cell fate decisions will be discussed.

Posttranscriptional Regulation of Mitochondrial Gene Expression

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The regulation of mammalian mitochondrial gene expression is primarily posttranscrptional. Processing and modification of the RNAs encoded in the primary polycistronic transcripts, and indeed the assembly of mitochondrial ribosomes, appears to occur in mitochondrial RNA granules, non-membrane delimited structures, that localize adjacent to mitochondrial nucleoids. Using immunoprecipitation and BioID, a proximity mapping tool, we identified a large toolbox of proteins in these RNA granules, many with uncharacterized functions, that likely have important roles in mitochondrial RNA transactions.

Maturation of most mitochondrial RNAs from the primary polycistronic transcripts occurs by cleavage of the tRNAs at the 5’ end by RNaseP and by RNaseZ at the 3’ end (the tRNA punctuation model). Processing of the remaining non-canonical mRNAs, without flanking tRNA sequences, has remained a mystery since the sequencing of human mtDNA four decades ago. We showed that depletion of FASTKD5, a member of the FASTK family of proteins, several of which localize to mitochondrial RNA granules, resulted in the accumulation of incompletely processed transcripts from precisely those open reading frames. This suggested that FASTKD5 could recognize specific RNAs and promote their processing. The phenotype was most pronounced for COX I, whose 5’ end was essentially unprocessed. This resulted in a severe decrease in the translation of this mRNA, and ultimately inhibited translation of all the mitochondrial mRNAs, suggesting that the mitochondrial ribosome cannot scan for the start AUG in the presence of a 5’ UTR. We speculate that loading of unprocessed mRNAs onto the ribosome results in a pool of ribosomes stalled at translation initiation that eventually halts translation and leads to cell death. We are testing the hypothesis that FASTKD5 can recognize, bind to, and process non-canonical precursor mRNAs and I will present our most recent results.

Regulation of mitochondrial gene expression

Peter Rehling
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Mitochondrial proteins are predominantly encoded in the nucleus and post-translationally imported into the organelle. The translocase of the outer mitochondrial membrane (TOM complex) mediates protein transport across the outer membrane. Transport across the inner membrane requires one of two translocases in the inner membrane (TIM complexes). A subset of the mitochondrial proteome however is encoded on mitochondrial DNA. These proteins are co-translationally exported across the inner membrane by Oxa1 and assemble with newly imported proteins into membrane protein complexes of the respiratory chain. In order to maintain mitochondrial function, the assembly of respiratory chain complexes from imported and mitochondria-encoded subunits has to be tightly regulated to adapted to cellular requirements. However, malfunction of these regulatory processes are linked to human disorders. To understand such regulatory processes, we have focused on the cytochrome c oxidase assembly process. Here, translational regulation is coupled to the assembly state of the enzyme complex. Our analyses provide new insight into the mechanism of inner mitochondrial membrane complex assembly.

Plant mitochondria RNA splicing as a key regulatory step in the biogenesis of the OXPHOS system

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Most mitochondria contain their own genomes (mtDNA, mitogenome) and intrinsic machineries for making their own RNAs and proteins. Although all mitochondria have evolved from a common ancestor, these have diverged considerably in different clades. The mtDNAs in plant cells are highly complex in structure, and their expression involves the transcription of numerous pre-RNAs that undergo extensive processing events, such as the removal of many intron sequences that reside within essential genes. Although the mt-introns generally belong to the group II-class, these often exhibit unconventional structural features, have lost their evolutionary-related maturase (MAT) splicing-cofactors, and follow unusual splicing pathways. Some undergone rearrangements within the mtDNAs, such as they are separately transcribed and are spliced ‘in-trans’. Their degeneracy was followed during the evolution by the acquisition of host-acting factors that belong to a diverse set of protein-families. These include proteins that are related to MATs and contain N-terminal mitochondrial localization signals, while others define RNA-binding protein families (e.g., CRMs, PORRs and PPRs), which are conserved between monocot and dicot plant species. The similarities between GIIs and nuclear-introns further suggest that the spliceosomal machineries have evolved from primordial GIIs. The analysis of plant mitochondria splicing provide us with important clues into the evolution and functions of their related spliceosomal machineries in the nucleus of eukaryotic cells. Here, we provide an update on the non-canonical MAT factors in angiosperm mitochondria, and summarize the current knowledge of their essential roles in regulating NAD1 expression and respiratory complex I biogenesis during early plant life.

Transcription, translation and degradation (Monday, November 14, 2022 14:00)

Ubiquitin-dependent and ubiquitin-independent steps in hypoxia-driven mitophagy

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Hypoxia, a reduction in the normal level of tissue oxygen tension, is implicated in pathophysiological conditions such as cardiac and cerebral ischemia and in growth of solid tumors. Oxygen deprivation, causes an aberrant electron transport through the electron transport chain of the mitochondria, culminating in the production of excessive reactive oxygen species (ROS). The resulting oxidative damage can lead to defective mitochondria or damaged proteins in the vicinity of mitochondria. Autophagy and the Ubiquitin-proteasome system (UPS) are two parallel yet interlinked pathways for the elimination of defective proteins and intact mitochondria. We observed that mitochondria undergo pervasive ubiquitination in response to hypoxia, suggesting a critical role of UPS in mitochondrial quality control. By contrast, previous reports largely attributed hypoxia-induced mitochondrial clearance to ubiquitin-independent receptor-mediated removal of damaged mitochondria. By isolating mitochondria from hypoxic cells and performing semi-quantitative proteomics, we confirmed that polyubiquitin conjugates at the mitochondria are largely K48- linked, implicating key involvement of the proteasome. In parallel, we also found that knocking out the ubiquitin-independent mitophagy receptors also impaired mitochondria clearance. By employing a mix of biochemical, genetic, and cellular approaches, we describe hypoxia-driven mitophagy occurring via two successive steps: an initial role of UPS in the maintenance of mitochondrial homeostasis followed by clearance of oxidative damaged mitochondria fragments mediated by ubiquitin-independent receptors.

Transcription, translation and degradation (Monday, November 14, 2022 14:00)

The story of yeast Lon protease in the mitochondrial DNA maintenance and assembly of respiratory chain complexes

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Mitochondria are crucial components of living cells. Changes in their functioning very often lead to severe human diseases, including myopathies, neurodegenerative diseases, and cancer. Also, aging has an origin in mitochondrial malfunction. Therefore, mitochondria possess their own quality control system consisting mainly of ATP-dependent proteases and chaperons. Lon is a unique ATP-dependent protease that not only degrades misfolded and non-assembled mitochondrial proteins, but also is responsible for regulating the level of several short-lived and regulatory ones. Here, we performed a proteomic mass

spectrometry analysis of purified yeast mitochondria on three yeast strains (wt, Lon overexpression and Lon deletion mutant) and detected 425 mitochondrial proteins with significantly changed concentration/protein level. The absence of functional Lon protease affects several crucial processes including mitochondrial translation, oxidative phosphorylation, biosynthesis of Fe/S clusters, TCA cycle as well as the mitochondrial genome maintenance. While the activity of respiratory complex II is only mildly reduced, remaining OXPHOS complexes III, IV and V are severely affected. This goes in line with the fact that Lon deletion mutant lacks most of the mitochondrial DNA sequence and thus also mitochondrial DNA-encoded proteins which in turn affects the assembly and functions of corresponding respiratory chain complexes. Despite the absence of the FO subunit of the complex V, the F1 subunit is assembled and exhibits an increased ATPase activity.

Acknowledgement

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Mitochondria and stress (Monday, November 14, 2022 16:25)

Cellular management of mitochondrial stress

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Mitochondria are semi-autonomous organelles that provide the energy and important building blocks required by our cells. The majority of mitochondrial proteins are made in the cytosol and then are transported and sorted to the proper submitochondrial localization using specialized molecular machines called translocases. Assembly of the TIM23 translocase in the mitochondrial inner membrane is an intricate process in which several accessory proteins are involved. However, the regulation of this process is not fully understood. Here we explore an unexpected role of OCIAD1 (ovarian carcinoma immunoreactive antigen-like protein 1) in the control of the TIM23 assembly. OCIAD1 was found to be upregulated in several carcinomas and also in some neurogenerative disorders. We report that it is predominantly localized in the outer mitochondrial membrane but it interacts with several inner membrane proteins, including subunits of the TIM23 translocase. Alterations of OCIAD1 result in regulation of the TIM23 assembly by altering the steady state levels of TIMM17A. Interestingly OCIAD1 undergoes a reciprocal regulation in response to a defective TIM23 translocase. Thus, we identify a novel regulatory loop with the critical role of OCIAD1 activated upon mitochondrial protein import and sorting deficiency.

Mitochondria and stress (Monday, November 14, 2022 16:25)

Upregulation of COX4-2 via HIF-1α and replicative stress and impaired nuclear DNA damage response in mitochondrial COX4-1 deficiency

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Cytochrome c oxidase (COX) is the final electron acceptor in the mitochondrial electron transfer chain. The mammalian COX is a dimeric complex composed of fourteen subunits. Primary COX deficiency is heterogenous group of disease, characterized by a wide range of clinical manifestations. In 2017, we reported a novel case of a 5-year-old child who carries a homozygous mutation (p.Lys101Asn) in the *COX4I1* gene. COX subunit 4 has two isoforms; COX4-1, the main subunit, is ubiquitously expressed in most mammalian tissues. COX4-2 is mainly expressed in the lungs, or under hypoxia and other stress conditions. Both isoforms play an important role in the activity, assembly, and regulation of COX.

We observed, that although the COX4-1 protein level is undetectable inpatient mitochondria, a significant residual COX activity remained. In this study we showed that COX4-2 levels are significantly up-regulated in COX4-1-deficient cells (patient's cells and COX4-1 knock-down cell) also in normoxia via stabilization and activation of HIF-1alpha. We therefore suggest that "pseudohypoxia" could be a compensatory mechanism for COX4-1 deficiency, explaining both the residual COX activity and the patient`s milder phenotype relative to other COX deficiencies.

Interestingly, the patient`s clinical manifestations were more similar to Fanconi anemia syndrome, which is characterized by bone-marrow failure, genome instability and cancer predisposition due to mutations in genes linked to DNA damage response. We demonstrated that also COX4-1 deficient cells are characterized by high levels of double-stranded breaks (DSBs), independantly of oxidative stress but induced by replicative stress. We also demonstrated that impaired DNA damage response, is the cause of replicative stress and accumulation of DSBs, which eventually leads to premature senescence.

Our findings suggest the involvement of the mitochondrial respiratory chain, with a direct link to COX4-1, in nuclear genome maintenance.

Mitochondria and stress (Monday, November 14, 2022 16:25)

UPR^{mt} scales mitochondrial network expansion with protein synthesis via mitochondrial import in Caenorhabditis elegans

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As organisms develop, individual cells generate mitochondria to fulfill physiological requirements. However, it remains unknown how mitochondrial network expansion is scaled to cell growth. The mitochondrial unfolded protein response (UPR^{mt}) is a signaling pathway mediated by the transcription factor ATFS-1 which harbors a mitochondrial targeting sequence (MTS). Here, using the model organism Caenorhabditis elegans we demonstrate that ATFS-1 mediates an adaptable mitochondrial network

expansion program that is active throughout normal development. Mitochondrial network expansion requires the relatively inefficient MTS in ATFS-1, which allows the transcription factor to be responsive to parameters that impact protein import capacity of the mitochondrial network. Increasing the strength of the ATFS-1 MTS impairs UPR^{mt} activity by increasing accumulation within mitochondria. Manipulations of TORC1 activity increase or decrease ATFS-1 activity in a manner that correlates with protein synthesis. Lastly, expression of mitochondrial-targeted GFP is sufficient to expand the muscle cell mitochondrial network in an ATFS-1-dependent manner. We propose that mitochondrial network expansion during development is an emergent property of the synthesis of highly expressed mitochondrial proteins that exclude ATFS-1 from mitochondrial import, causing UPR^{mt} activation.

Mitochondria and stress (Monday, November 14, 2022 16:25)

An inventory of the mitochondrial proteins affected by deficiency of the HSP60/10 chaperone complex; substrates and band waggoneers

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Heat-shock protein 60 (HSP60) is a mitochondrial molecular chaperone that together with its co-chaperone HSP10 forms ring complexes. The HSP60/HSP10 complex functions as an isolation chamber to promote and assist folding and conformational maintenance of proteins in the mitochondrial matrix space. Loss of the complex is lethal and mutations in HSP60 and HSP10 cause rare neurodevelopmental disorders with impaired myelination as a common characteristic.

We have in a previous study shown that more than 250 mitochondrial proteins interact with Hsp60. In the present study we validate which of the interactors are affected by HSP60/HSP10 complex deficiency. Proteomic and RNASeq analysis of a HEK cell system for inducible expression of a dominant-negative mutant of HSP60 (DN-HSP60) shows that the levels of more than 250 matrix and inner membrane proteins that use the TIM23 import system decrease significantly with induction time while the encoding transcripts are unaffected. This is consistent with the notion that folding of these proteins is impaired by HSP60/10 complex deficiency resulting in their proteolytic degradation. Affected proteins cover the major metabolic pathways, the respiratory chain, and mitochondrial gene expression. Analysis of the levels of a series of metabolites pictures a broad derangement of metabolic pathways. There is no indication of specific protein structure properties distinguishing affected from unaffected proteins. Surprisingly, only about half of the previously identified interactors is affected indicating that also proteins that fold independent of the complex interact.

RNASeq analysis revealed a number of transcriptional responses triggered by HSP60/10 deficency including the integrated stress response (ISR), however, the mitochondrial unfolded protein response was not noticeably activated. Induction of the DN-HSP60 variant triggered regulation of transcripts encoding extramitochondrial enzymes that are involved in cholesterol synthesis. This finding may rationalize the hypomyelination phenotype observed in patients with mutations in HSP60 and HSP10.

mtHsp70 converts mitochondrial proteostasis distress into impaired protein import

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Structure and function of mitochondria critically depend on import of over 1000 different proteins from the cytosol. Protein import into mitochondria is also used as a sensitive mechanism to monitor the functionality of mitochondria and impaired protein import initiates different well-characterized cellular programs that rescue or remove dysfunctional mitochondria. However, the molecular mechanism that underlies the initial reduction of protein import into defective mitochondria remained unclear. Here, we uncovered that mtHsp70, the major mitochondrial chaperone that is involved in both folding and import of proteins, is the key sensor of mitochondrial fitness. We found that the distribution of mtHsp70 between the TIM23 complex at the inner membrane, where it is involved in protein import, and the matrix, where it is involved in folding of proteins and prevention of their aggregation, is tightly balanced and is used to regulate the efficiency of protein import. During early mitochondrial stress, before rescue programs are initiated and membrane potential is affected, mtHsp70-dependent import was specifically impaired and association of mtHsp70 with the import complex reduced. Analysis of the conformation of mtHsp70 in intact mitochondria on the single molecule level revealed that the majority of the chaperone is present in a substrate-bound state, even under non-stress conditions. We propose that the availability of free mtHsp70 limits protein import into mitochondria during stress.

VDAC1 overexpression in diseases: from cancer to diabetes, neurodegenerative and autoimmune diseases: a common target and treatment

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The voltage-dependent anion channel 1 (VDAC1) protein stands at the crossroads between mitochondrial energy production and metabolism, Ca2+ homeostasis, apoptosis execution and regulation, and other cell stress-associated processes. VDAC1 as the mitochondrial gatekeeper serves as a hub protein interacting with over 100 proteins, allowing it to mediate and regulate the integration of mitochondrial functions with cellular activities. Recently, we demonstrated that apoptosis induction leads to VDAC1 overexpression and oligomerization, regardless of the cell type or apoptosis inducer used. Thus, we proposed the formation of a large protein-conducting channel within a VDAC1 homo-oligomer serving as the pro-apoptotic protein release route and, thus, apoptosis. Moreover, VDAC1 overexpression, its oligomerization and apoptosis induction were demonstrated to be associated with different diseases. These include neurodegenerative disease as ALS and Alzheimer's, type 2 diabetes, lupus, colitis, acute liver injury, rheumatoid arthritis, spinal cord injury, myocardia of humans and rats and COVID-19 patients' T cells. Moreover, in all these diseases, VBIT-4 or VBIT-12, newly developed inhibitors of VDAC1 oligomerization, prevent mitochondria dysfunction and apoptosis cell death and associated processes induced by the overexpressed VDAC1. These results support the tight coupling between VDAC1 overexpression, VDAC1 oligomerization, apoptosis induction, and pathological states. Thus, our studies, followed by others, suggest that overexpression of VDAC1 is a common threat in diabetes; neurodegenerative, cardiac, and autoimmune diseases, and others. As such, inhibiting VDAC1 overexpression and/or its oligomerization, as an early stage of apoptosis represents an effective target to treat these diseases.

Sex-specific and tissue-specific regulation of mitochondrial functions and their relationships to clinical traits

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We study mitochondrial functions and their relationships to common metabolic and cardiovascular traits by examining how they are perturbed by natural genetic variation. Thus, we have examined mitochondrial functions in hearts, white adipose tissues and livers of 100 genetically diverse inbred strains of mice that have been characterized for a variety of relevant clinical traits. To understand the molecular factors contributing to the genetic variations, we have also performed global transcriptomic or metabolomic analyses of each tissue in all of the strains. We find that mitochondrial functions are independently controlled in each of these tissues. For example, expression of the mitochondrial complex I protein, Ndufv4, varies among the strains in adipose but not other tissues due to a regulatory locus on mouse Chromosome 17. This variation is strongly and causally associated with mitochondrial functions and DNA levels as well as obesity, insulin resistance, and plasma lipids. Similarly, mitochondrial gene expression and functions in heart vary among the strains, independently of the other tissues, and they associated with a common form of heart failure. Mitochondrial functions in all three tissues also differ between sexes. For example, in heart, males have more mitochondria than females, in both mice and humans, and this appears to explain in part the greater susceptibility of females to a common form of heart failure. In white adipose, on the other hand, females have higher levels of mitochondria than males, in both mice and humans, and this is associated with resistance to obesity and insulin resistance.

Mitochondrial DNA as a regulator of host immunity and the gut microbiota

Tal Yardeni

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Both mitochondrial DNA (mtDNA) lineages and the gut microbiota have been correlated with altered risk for a variety of human diseases. However, the mechanisms by which mtDNA variation and the gut microbiota modulate disease risk remains unknown. Our hypothesis is that *both the gut microbiota and the immune system are modulated by the mitochondrial genome, in part through mitochondrial reactive oxygen species (mROS) production, forming a critical link between the gut microbiota and disease initiation and progression.*

Our studies showed significant differences in gut microbiota in our conplastic mice which differ in their mtDNA lineages. Further, the transfer of the gut microbiota from a host of one mitochondrial genotype to a host of different mitochondrial genotypes shifted the gut microbiota composition toward that of the recipient animal. Moreover, we showed that host mROS levels modulated the composition of the gut microbiota.

Those conplastic mice also exhibit markedly different capacities to sustain melanoma tumor growth. Relative to control mtDNA ($mtDNA^{B6}$) mice, the mice harboring NZB mtDNAs ($mtDNA^{NZB}$) have strong anti-tumor immune response while those with 129 mtDNA ($mtDNA^{129}$) are the opposite. When we reduced mROS by expression of mitochondrial catalase (mCAT)Tg in eight tissue-specific experiments. Only in the hematopoietic cells the reduction of mROS changed the gut microbiota and obviated the anti-tumor effects on the $mtDNA^{NZB}$ and $mtDNA^{B6}$ mice. *These observations suggest that melanoma severity, and gut microbiota are modulated by the mtDNA's regulation of mROS production in host immune cells, pointing to new potential pathways for melanoma therapeutic development*.

Mitochondria related diseases (Tuesday, November 15, 2022 09:00)

Mitochondrial augmentation technology (MAT): mitochondrial enhancement of the hematopoietic system

Natalie Yivgi Ohana
Research, Minovia Therapeutics, Tirat Hacarmel, Israel

Mitochondrial Augmentation Technology (MAT) is a platform technology designed for enriching cells with healthy exogenous mitochondria.

To address the underlying cause of Primary mtDNA disorders, delivery and persistence of exogenous mtDNA is necessary in order to achieve efficacy in diseased target organs.

Our first product is composed of hematopoietic stem and progenitor cells (HSPCs) enriched with allogeneic mitochondria. While maintaining their stemness state, HSPCs are quiescent and mostly use glycolysis to produce ATP, yet they require high levels of mitochondrial activity to differentiate and proliferate into the various hematopoietic lineages. Using allogenic placental-derived mitochondria and novel analytical methods, we are exploring parameters affecting persistence of exogenous mtDNA after MAT and the effect of co-existence of 2 different haplogroups on cell functionality. For this purpose we have generated a bank of qualified mitochondria of numerous haplogroups and are using these to enrich HSPCs with mitochondria of multiple haplogroups. As a model for mitochondrial diseases, we are using patient-derived Lymphoblastic Cell Lines (LCL) with different heteroplasmy levels, that allows us to study the ability of MAT to rescue disease phenotype. Single-cell methodologies such as ATP-dependent protein synthesis have demonstrated that higher levels of augmentation enable higher protein synthesis levels.

In addition, we are intrigued by the recent evidence suggesting that immunological factors play a significant role in PMD pathology even in the absent of clear hematological or immunological manifestations. We are currently exploring mechanisms by which MAT using HSPCs may treat PMD with significant and durable benefit by altering the immune system.

Mitochondria related diseases (Tuesday, November 15, 2022 09:00)

Mitochondrial regulation of activity homeostasis in central neural circuits

Inna Slutsky
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Maintaining average activity level within a set-point range constitutes a fundamental property of central neural circuits. Accumulated evidence suggests that mean firing rate (MFR) represents a physiological variable regulated by homeostatic systems in central neural circuits. However, some central questions have remained open. What are the mechanisms that establish the specific values of MFR set-points? Are MFR set-points fixed or adjustable in central neural circuits? If they are adjustable, do separate mechanisms control negative feedback responses and MFR set-point value? And finally, whether re-adjustment of dysregulated firing set-points may provide a new conceptual way to treat brain disorders associated with pathological set points? I will present our approach integrating genome-scale metabolic modeling and experimental study of neuronal homeostasis to predict novel homeostatic regulators. Next, I will describe mitochondrial molecules that play a key role in the regulation of activity homeostasis in neural circuits. Finally, I will provide the evidence for a new conceptual strategy to suppress pathological brain activity by lowering firing set-points.

The metabolic and epigenetic effects associated with chronic IF1 loss

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Mitochondria play many roles in cell biology that go beyond bioenergetics. For example, while changes in the tricarboxylic acid (TCA) cycle modulate the epigenome and gene expression, maintenance of the mitochondrial membrane potential (ΔΨm) influences proliferation. In a previous study, we showed that loss of ATPIF1 (IF1), a natural inhibitor of the hydrolytic activity of the ATP synthase, maintained the ΔΨm and cell proliferation even in the complete absence of mitochondrial DNA (rho0) – a state that causes cell cycle arrest in wild-type (WT) cells. How exactly the ΔΨm impacts proliferation remains unclear, but it was proposed to involve redox signaling. Here, we used genomics approaches to gain molecular insights into how IF1 loss allows rho0 cells to proliferate. Using RNA-seq, we found only 680 differentially expressed genes (DEGs) in IF1KO rho0 cells, while over 1,200 were identified in the WT rho0. As expected, some of the upregulated DEGs were involved in cell cycle regulation. Most notably, over 80% DEGs in IF1KO were repressed, which coincided with nuclear DNA hypermethylation. Surprisingly, loss of IF1 per se led this phenotype, with mtDNA depletion having no further effect on DNA methylation. Analysis of IF1KO cells (with mtDNA) revealed significant remodeling of the metabolome and transcriptome, largely involving glucose metabolism and the pentose phosphate pathway (PPP). Interestingly, despite no signs of mitochondrial stress at the biochemical or ultrastructural levels, cells depleted of IF1 had transcriptional changes akin to mitochondrial dysfunction. These data suggest that while subtle, the effects of chronic IF1 depletion per se initiate a cascade of metabolic and transcriptional remodeling that seemingly prepares the cells to better handle subsequent stress, including that provided by mtDNA loss. Currently, we are testing whether and how the metabolic changes caused by chronic IF1 loss drive the DNA epigenetic phenotype thereby changing the normal response to mtDNA depletion.

Structural insights into the mechanisms of Fe/S protein biogenesis in mitochondria

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Iron-sulfur (Fe/S) proteins are involved in numerous important cellular processes such as respiration, metabolism, genome maintenance, protein translation and antiviral response. The synthesis of Fe/S clusters and their assembly into apoproteins in (non-green) eukaryotes is a complex process involving more than 30 proteins located in mitochondria and cytosol. Biogenesis of mitochondrial [2Fe-2S] and [4Fe-4S] proteins is accomplished by the iron-sulfur cluster assembly (ISC) machinery which was inherited from bacteria during evolution [1-3]. Cytosolic and nuclear Fe/S protein assembly also depends on the function of this machinery, yet additionally requires the mitochondrial ABC exporter ABCB7 and the cytosolic iron-sulfur protein assembly (CIA) machinery [4]. Interestingly, mitochondrial Fe/S protein biogenesis co-evolved with the existence of the entire organelle, defining this process as both the minimal and essential function of mitochondria [5]. A combination of in vivo and in vitro studies have generated a decent picture of the general outline of Fe/S protein biogenesis. The detailed molecular mechanisms underlying the individual reaction steps are currently under investigation by using cell biological, biochemical, spectroscopic, and structural approaches. The presentation will provide some of our recent insights into the molecular mechanisms of [2Fe-2S] and [4Fe-4S] cluster assembly in mitochondria, and the multiple functions of the two human mitochondrial [2Fe-2S] ferredoxins FDX1 and FDX2. These mechanistic insights may eventually help improving our molecular understanding of the biochemical consequences of numerous “Fe/S diseases” linked to most ISC gene mutations.

Reviews:
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3. Lill, R. Biol Chem, 2020. 401(6-7): p. 855-876.
4. Paul, V.D. and R. Lill. BBA, 2015. 1853(6): p. 1528-1539.
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Mitochondrial metabolism (Tuesday, November 15, 2022 18:00)

Targeting metabolic vulnerabilities of TCA cycle deficient tumors

Eyal Gottlieb
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Metabolic rewiring is a hallmark of cancer and has been the focus of considerable research interest in the last decades, particularly because of the potential to identify druggable metabolic vulnerabilities specific for cancer cells. Although several metabolic features have been associated with tumorigenesis, only a few targets have materialized into efficient anti-metabolic strategies to treat cancer. Succinate dehydrogenase (SDH) and fumarate hydrates (FH) are TCA cycle enzymes that are also bona fide tumor suppressor genes which are deficient in several types of cancers. Currently, there are no specific therapeutic approaches to treat patients with SDH- or FH-deficient tumors. We screened for synthetic lethal targets for TCA cycle deficient tumors. These tumor cells rewire their metabolism towards glycolysis, exposing a potential targetable liability. Indeed, we demonstrated that drugs that effectively block glucose uptake are extremely effective in killing TCA cycle deficient cells by inducing energy depletion. These effects were specific for tumors deprived of TCA cycle enzymes, hence these compounds may provide a sufficient therapeutic opportunity to treat those cancers.

Mitochondrial metabolism (Tuesday, November 15, 2022 18:00)

T cells adaptation to restrained oxidative-phosphorylation (OXPHOS) capacity

Michael Berger[1], Amijai Saragovi[1], Ifat Abramovich[2], Eyal Gottlieb[2], Magdalena Huber[3]
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$CD8^+$ T cells' metabolic programs are critical for their function and fate decisions. As the metabolic hub of the cell, the mitochondria play a crucial role in T cell-mediated immunity. However, the mechanisms by which mitochondria govern T cell responses and cellular fate are not completely understood. Recently, we demonstrated that mitochondrial oxidative phosphorylation (OXPHOS) is vital for $CD8^+$ T cell activation by supporting matrix substrate-level phosphorylation, independently from ATP synthesis by complex V. Here, to unveil the underlying mechanisms adapted by $CD8^+$ T cells to function independently from mitochondrial ATP, we tested how depleting mitochondrial ATP from the $CD8^+$ T cells' cytoplasmic compartment affect their activation by generating a T cell-specific ADP/ATP translocase-2 knockout ($Ant2^{KO}$) model. Remarkably, we found that Ant2 deficiency improved $CD8^+$ T cell activation and effector functions. Following these findings, we demonstrated that naïve $Ant2^{KO}$ $CD8^+$ T cells overcome restrained OXPHOS capacity by increasing their mitochondrial biomass, spare respiratory capacity, and through the induction of selective anabolic pathways. Specifically, these pathways include aerobic glycolysis, TCA-cycle activity, and aspartate biosynthesis, ROS production, nucleotides biosynthesis, and proline biosynthesis. Since the role of proline biosynthesis pathway in T cell activation is unknown, we performed a study showing that similarly to naïve $Ant2^{KO}$ $CD8^+$ T cells, activated wild-type $CD8^+$ T cells also engage in proline biosynthesis, to support the increased demand for oxidation reactions within the mitochondria when OXPHOS does not meet those demands. Overall, our study provides a better understanding of the metabolic programs adapted by $CD8^+$ T cells to support rapid proliferation and effector functions under conditions such as low oxygen tension and metabolic competition.

Mitochondrial metabolism (Tuesday, November 15, 2022 18:00)

MTHFD2 promoted metabolic reprograming is supported by the mitochondrial chaperone HSPD1 in lung cancer

Shani Gabbay, Barak Rotblat
Life Science, Ben-Gurion University, Beer Sheva, Israel

Metabolic reprogramming is a hallmark of cancer facilitating cell adaptation to metabolic stress and promoting cell growth. One carbon metabolism (1CM) is one of the pathways which are involved in such metabolic reprogramming. The folate cycle, one of the two coupled cycles in the 1CM, promotes the synthesis of purines and NADH and thus, supports proliferation. MTHFD2 is a mitochondrial enzyme participating in the folate cycle and has been found to be highly expressed in lung cancer where it promotes cell growth, migration, and generation of antioxidants. Similar to most mitochondrial proteins, MTHFD2 is synthesized in the cytosol and is translocated to the mitochondria in its unfolded state. The mechanism promoting MTHFD2 folding in the mitochondria state is unclear. Previously, our lab has shown that among the different mitochondrial chaperones, the expression HSPD1 (a family member of HSP60) has the highest correlation with MTHFD2. We hypothesize that the folding of MTHFD2 in the mitochondria depends on HSPD1 and therefore, HSPD1 supports metabolic reprogramming in cases where MTHFD2 is induced. MTHFD2 is highly expressed in A549 adenocarcinoma cells and H358 non-small cell lung carcinoma. Our initial data demonstrate that MTHFD2 expression is dependent upon HSPD1 at the protein but not mRNA level. Additionally, we found that replenishing full medium to WT

cells, following serum and glucose starvation, leads to increased MTHFD2 levels and we plan to use this system, together with the HSPD1 KD cells, to test the importance of HSPD1 in MTHFD2-mediated metabolic reprogramming. Furthermore, we aim to test the effect of HSPD1 on one-carbon metabolism and tumorigenicity in lung cancer cells. Eventually, this study will reveal the role of HSPD1 in metabolic reprograming through MTHFD2 induction in lung cancer cells and will set the stage for future studies of the role of mitochondrial chaperone-client interactions in metabolic reprogramming.

More Mitochondrial Metabolism (Wednesday, November 16, 2022 09:00)

Inhibition of Complex-V ATP hydrolytic activity restores energy homeostasis in mitochondrial pathologies

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Mitochondrial ATP synthase (Complex V; CV) can hydrolyze ATP in response to mitochondrial depolarization. The mitochondrial protein, ATPIF1 can inhibit CV-ATP hydrolysis while also reducing synthesis rate. Here we used in-silico and in vitro analyses to find small molecules that have high affinity to the CV-ATPIF1 binding groove and identified (+)-epicatechin as a selective inhibitor of hydrolysis while not affecting synthesis. In reconstitution assays we find that (+)-epicatechin competes with ATPIF1 on its binding site to the F1 head of CV. In cells with CIII deficiency, we show that inhibition of CV hydrolytic activity is sufficient to restore ATP content and improve cellular proliferation. Inhibition of CV-ATP hydrolysis in a model of Duchenne Muscular Dystrophy is sufficient to improve muscle force without any increase in mitochondrial content. We conclude that the impact of compromised mitochondrial respiration can be lessened using hydrolysis-selective inhibitors of CV.

Metabolic and physiological roles of the mitochondrial $Na^+/Ca2^+$ exchanger NCLX

Israel Sekler
Physiology and Cell Biology, Ben-Gurion University, Beer-Sheba, Israel

The mitochondria are not only the energy supplier but also a major Ca2+ signaling hub of the cell. Powered by the mitochondrial membrane potential Ca2+ permeates the mitochondria via the Ca2+ channel MCU followed by Ca2+ efflux by the 3Na+/Ca2+ exchanger, NCLX. We study the mode of regulation and physiological roles of NCLX. In the first part of my talk, I will describe a new mode of regulation of NCLX by the casein kinase 2 (CK2). We find that phosphorylation of NCLX is CK2 is required for its activation and for tuning an ultrafast mode of axonal initial segment plasticity. We also find that caffeine of required to activate NCLX in hippocampal neurons by inhibiting phosphodiesterase 2 (PDE2), thereby controlling PKA-dependent NCLX phosphorylation and activation. PDE2-dependent regulation of NCLX dictates neuronal fate following excitotoxic insult and controls new object learning in mice. Finally, we interrogate the hepatic role of NCLX. We find that NCLX is essential for mitochondrial Ca2+ efflux in hepatocytes. Importantly NCLX is required for mitochondrial Ca2+ oscillation triggered by the hepatic hormones Glucagon and Vasopressin. Consistent with these results, we find that NCLX is required for glucagon-dependent gluconeogenesis both in hepatocytes culture and in vivo in mice.

The pyruvate dehydrogenase complex regulates mitochondrial matrix protein phosphorylation and mitophagic selectivity, independent of its catalytic activity

Hagai Abeliovich
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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 mutations. The culling of defective mitochondria is thought to delay the onset of aging symptoms, and defects in mitophagy have been linked to late onset disorders such as Parkinson's disease and type II diabetes. We previously demonstrated that different mitochondrial matrix proteins undergo mitophagy at different rates, and that mitochondrial matrix protein phosphorylation and dephosphorylation can generate a segregation principle that would couple with mitochondrial fission and fusion dynamics to selectively degrade sub-sets of mitochondrial proteins. We now demonstrate a role for the pyruvate dehydrogenase complex (PDC) as a signaling nexus in this regulatory network. We demonstrate that the PDC controls the activities of its cognate kinases and phosphatases towards other mitochondrial matrix proteins, and that this novel function can be uncoupled from the pyruvate dehydrogenase catalytic function itself. Our data support a model where the PDC functions in a structural role to allosterically regulate its associated kinases and phosphatases towards 'third party' proteins in the mitochondrial matrix, suggesting a possible regulatory link between the levels of central mitochondrial metabolites and the regulation of mitophagic selectivity.

MTCH2 at the interface between homeostasis and apoptosis

Atan Gross
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Mitochondria are highly dynamic organelles that play fundamental roles in pivotal cellular processes including energy production, metabolism, and apoptosis. We are interested in understanding how these different mitochondrial processes are coordinated to respond to cellular stress. Many of our studies are focused on a novel mitochondrial protein named mitochondrial carrier homolog 2 (MTCH2) that mediates the response of mitochondria to stress signals initiating at the plasma membrane or at the nucleus. In the TNFa/Fas-death receptor pathway, MTCH2 acts as a receptor-like protein for BH3-only BID, important for cytochrome c release and for Fas-induced liver apoptosis in vivo. On the other hand, in the DNA damage pathway, MTCH2 acts as the down-stream effector of the ATM kinase/BID pathway in haematopoietic stem cells (HSCs), controlling HSC quiescence and survival via regulation of mitochondria metabolism. Recently we revealed that MTCH2 also plays a role in regulating mitochondrial fusion/elongation, which is important in driving the exit from naïve pluripotency in embryonic stem cells (ESCs). Thus, MTCH2 is an important regulator of mitochondria morphology and metabolism acting at the interface between homeostasis and apoptosis. Determining MTCH2's exact mechanism of action may lead to deciphering the mechanism by which BID, and perhaps other BCL-2 family members, regulate apoptosis.

p38 pathway links cholesterol metabolism with mitochondrial function and dysfunction

Amir Sapir
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In eukaryotes, cholesterol plays a pivotal role in membrane integrity and dynamics and is the processor for hormones and vitamins that coordinate development, growth, and reproduction. Recently, we have discovered that in contrast to vertebrates nematodes and most arthropods are cholesterol auxotrophs. The cholesterol auxotrophy of the nematode Caenorhabditis elegans (C. elegans) enables us to use this model organism as a cholesterol-defined experimental system for the study of cholesterol metabolism in animals and humans. Testing the hypothesis that changes in the level of cholesterol can elicit stress responses, we screen through an array of stress reports in C. elegans grown in different concentrations of cholesterol. In the screen, we identified two p38-mediated signaling axes that are activated in a concentration-dependent manner upon decreasing cholesterol levels. One of these p38 axes, the DLK-1/SEK-3/PMK-3 stress pathway, was previously shown to be activated upon mitochondrial stress in a UPRmt-independent manner. Thus, our finding links cholesterol depletion with a UPRmt-independent mitochondrial stress response. Downstream to cholesterol, we found that the main steroid hormone of C. elegans, Dafachronic Acid, cannot suppress the mitochondrial stress response activated by decreasing cholesterol levels. This result suggests a novel axis of a yet-to-be-identified cholesterol byproduct whose function is required for the proper integrity and activity of the mitochondria. Currently, we are assessing the effect of cholesterol depletion on mitochondrial function and dysfunction in vivo in different physiological conditions such as development, stress, and aging. In addition to the known requirement of cholesterol in the mitochondria for the synthesis of steroid hormones, our results demonstrate that cholesterol plays a novel and critical role in maintaining mitochondrial homeostasis and function.

Metabolism, cellular decisions and the language that unites them

Jared Rutter
Howard Hughes Medical Institute and Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, UT, USA

Mitochondria are dynamic and complex organelles that play a central role in all aspects of biology, including energy production, intermediary metabolism, and apoptosis. These broad cellular functions also place mitochondria as a central player in human health and disease. We have focused recently on deciphering the biochemical and cellular functions of conserved uncharacterized mitochondrial proteins. This has revealed new mechanisms for several critical aspects of mitochondrial function, including the Mitochondrial Pyruvate Carrier (MPC), which is required for efficient mitochondrial pyruvate uptake. By perturbing the metabolic program of cells, MPC manipulation profoundly affects cellular decisions impacting stem cell homeostasis and uncontrolled proliferation. This observation suggests that metabolism is not a passive bystander in determining the behavior of cells, but instead plays a decisive role. One of our current areas of focus, which will be discussed, is to determine the mechanisms whereby metabolism and metabolites affect behaviors via direct modulation of proteins involved in signaling, transcription and other regulatory mechanisms. As we discover these mechanisms, which we hypothesize to be extensive within biology, we will be enabled to impinge on such phenomena for therapeutic benefit in many disease states.