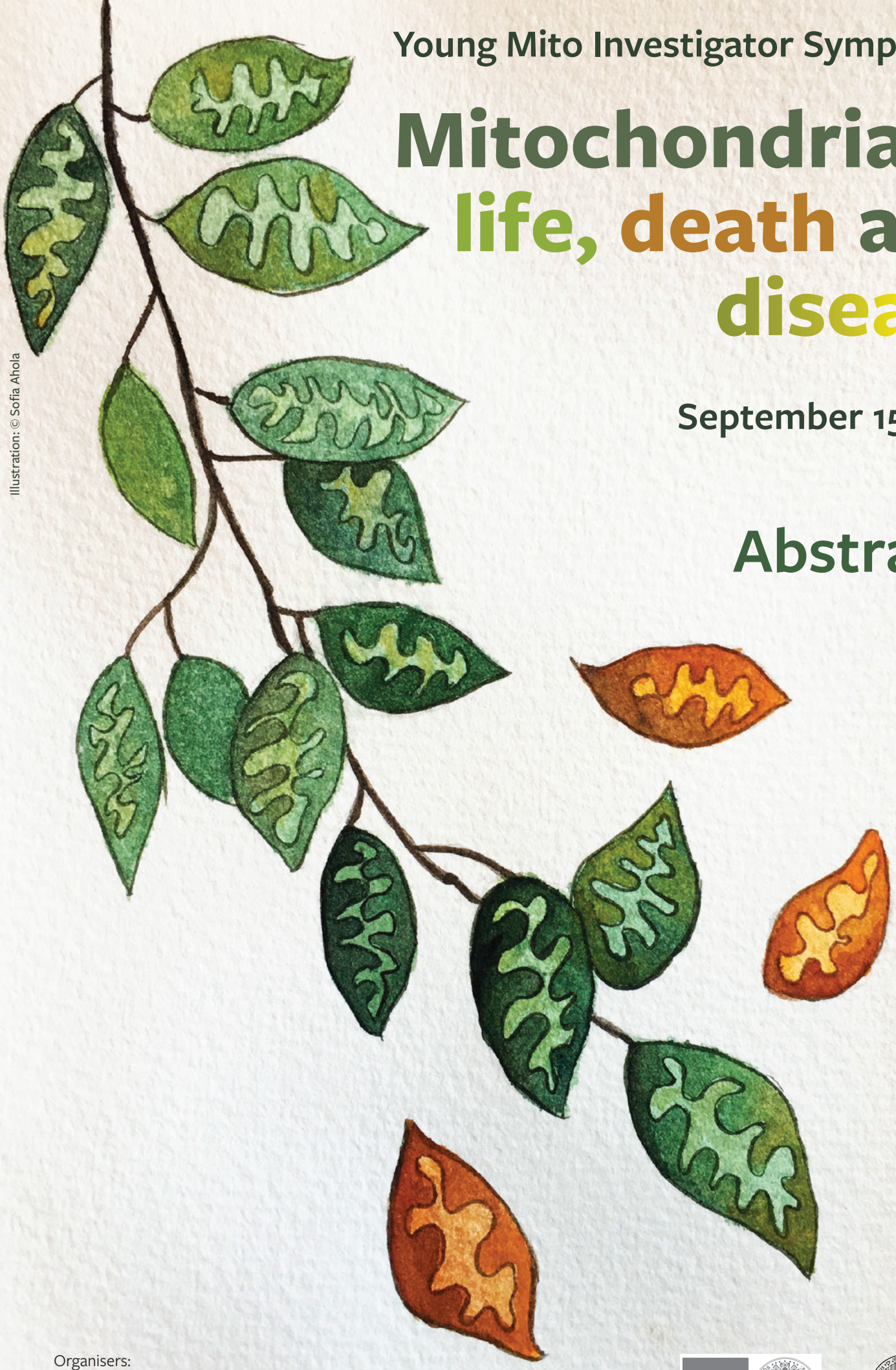
Young Mito Investigator Symposium

Mitochondria in life, death and disease

September 15th–17th

Abstracts



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Investigating sub-mitochondrial localisation of translation machinery components

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The mitochondrial translation machinery comprises numerous protein- and RNA-components and sub-assemblies which work in concert to facilitate mitochondrial translation. Recently, it was established that translation does not occur homogeneously throughout the mitochondrial network, but a steep gradient of translational activity can readily be observed from the vicinity of the nucleus to the periphery of the cell. Interestingly, we find that this does not correlate with restricted supply of single components of the ribosome or messenger RNA in the cell periphery; however, we do not know if the individual components from functional units in areas with low translational activity. Here, we investigate the relationship of single components of the translational machinery on a nano-scale level using multi-colour stimulated emission depletion (STED) microscopy.

Mitochondrial ribosomal proteins developed unconventional mitochondrial targeting signals due to structural constraints

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Mitochondrial ribosomes are complex molecular machines indispensable for respiration. Their assembly involves the import of several dozens of mitochondrial ribosomal proteins (MRPs), encoded in the nuclear genome, to the mitochondrial matrix. Available proteomic and structural data as well as computational predictions indicate that up to 25% of MRPs do not have a conventional N-terminal mitochondrial targeting signal (MTS). We characterized a set of 15 yeast MRPs *in vivo* and showed that 30% of them use exclusively internal mitochondrial targeting signals. We isolated a novel internal targeting signal from the conserved MRP Mrp17 (bS6). The Mrp17 targeting signal shares some properties as well as import components with conventional MTS-containing proteins but is not reliably predicted indicating that mitochondrial protein targeting is more versatile than expected. We hypothesize that internal targeting signals arose in MRPs when the N-terminus extension was constrained by ribosome assembly interfaces.

F-ATP synthase mutagenesis and its implication on formation and regulation of the Permeability Transition Pore

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The mitochondrial permeability transition pore (PTP) is a Ca^{2+}-activated channel that plays a key role in cell death. F-ATP synthase is a leading candidate to generate the PTP but the mechanism(s) leading to channel formation remain undefined. To shed light on the structural requirements for PTP formation we tested cells ablated for the F-ATP synthase dimerization subunit g (that also lack subunit e) and found that they did not show PTP channel opening in intact cells or patch-clamped mitoplasts unless the adenine nucleotide translocator (ANT)-specific ligand, atractylate, was added. This indicates that subunits g and e are strictly necessary for a functional PTP and that the contribution of ANT emerges in the absence of an assembled F-ATP synthase, suggesting that these two players mediate distinct permeability transition pathways. Thiol oxidation facilitates PTP opening, yet the targets and molecular mechanisms still await a definition. We identified on the F-ATP synthase one of the critical thiols that modulates PTP opening by oxidation. The C141 of the oligomycin sensitivity conferral protein (OSCP) subunit contributes to mediate the inducing effect of the thiol reagent diamide to the PTP and this occurs when cyclophilin D (CyPD) has been ablated. This last finding suggests that CyPD, a well-known PTP activator, might exert in some cases a protective role, shielding the OSCP C141 from oxidation. Altogether these data strongly support the primary role of the F-ATP synthase in PTP formation and shed light on structural/functional features that switch the energy-conserving enzyme to an energy-dissipating system.

Bacterial outer membrane vesicles induce mitochondrial dysfunction and intrinsic apoptosis

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Outer membrane vesicles (OMVs) released by Gram-negative bacteria contribute to infectious diseases. The OMV-associated endotoxin activates cytoplasmic receptors triggering macrophage death, inflammation, and sepsis. We have recently shown that OMVs from pathogenic bacteria, such as *N. gonorrhoeae*, deliver protein toxins to mitochondria to kill macrophages. How OMVs that target mitochondria trigger macrophage death and whether inflammation is induced or not remain unclear. We now have deciphered that OMVs derived from pathogenic bacteria cause mitochondria dysfunction, which activates intrinsic apoptosis and the NLRP3 inflammasome. Mechanistically, mitochondrial dysfunction reduces host cell protein translation and triggers the loss of the short-lived pro-survival BCL-2 family member, MCL-1, thereby unleashing the mitochondrial death factor, BAK. BAK-induced apoptosis in OMV treated macrophages and potassium ion efflux trigger NLRP3-dependent secretion of the inflammatory cytokine, IL-1β. Importantly, mice deficient in intrinsic apoptosis contain reduced IL-1β serum levels in response to *N. gonorrhoeae* OMVs. These findings identify OMVs as activators of the host cell mitochondrial apoptosis machinery to trigger both macrophage death and inflammasome activation. Collectively, our findings suggest a host immune surveillance mechanism that monitors mitochondrial health to detect pathogenic bacteria.

Deciphering the mitochondrial quality control mechanism in the parasite *Trypanosoma brucei*

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Mitochondrial quality control (MQC) is the network of pathways by which eukaryotic cells monitor and maintain the function of their mitochondria. We study this process in the protist *Trypanosoma brucei*. Due to evolutionary divergence from opisthokonts, *T. brucei* has many unique features. With relevance here, it has a single network-like mitochondrion with a single unit mitochondrial genome, preventing the elimination of individual dysfunctional mitochondria as in some other organisms, and transcription in *T. brucei* is exclusively polycistronic, making transcriptional regulation of MQC essentially impossible. Other than ubiquitin and the proteasome, orthologues of many common opistokont MQC factors are absent in *T. brucei.* Many examples of convergent evolution have been discovered in *T. brucei*, particularly concerning mitochondrial biogenesis, and we expect the same to be the case for mechanisms governing MQC.

We will show data demonstrating the existence of a MQC pathway in *T. brucei* triggered when mitochondrial protein import is blocked, where the endpoint is the proteasomal degradation specifically of mislocalised mitochondrial proteins. We show the proteasome and ribosome are recruited to the mitochondrion upon the induction of this import defect, along with several trypanosomatid-specific proteins which we show are required for this MQC pathway. These candidates are being investigated as to their roles within a putative ubiquitination cascade. Of particular interest is a nuclearly-localised protein with a ubiquitin-like domain, which appears to be released into the cytoplasm upon the induction of this mitochondrial protein import defect. This process is required for the MQC mechanism to act.

Unconventional protein import pathways for dually localised proteins present in the intermembrane space

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The mitochondrial intermembrane space (IMS) is an important compartment for maintaining mitochondrial and cellular redox balance. Aberrant protein targeting and function within this compartment can have serious disease implications due to redox issues such as the build-up of reactive oxygen species generated by the respiratory complexes of the inner membrane. Glutathione peroxidases (Gpxs) are present throughout the cell and work to sense and scavenge H_2O_2. In yeast, Gpx3 is found in both the cytosol, where it acts in conjunction with the transcription factor Yap1 as a sensor for H_2O_2, and within the mitochondrial IMS where its role is less clear but may involve limiting lipid peroxidation. How such dually localised proteins (of which there are an ever-increasing number in both yeast and humans) are imported into the IMS remains relatively unexplored but may provide valuable insights into certain disease progressions. Gpx3 does not appear to follow a traditional import route into the IMS hinting at the presence of as yet unknown mechanisms and proteins that may play a significant role in mitochondrial function by facilitating the import, and retention, of dually localised proteins lacking a traditional mitochondrial targeting signal.

Reduced expression of mitochondrial complex I subunit NDUFS2 does not impact healthspan in mice

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Aging in mammals leads to reduction in genes encoding the 45-subunit mitochondrial electron transport chain (ETC) complex I. It has been hypothesized that normal aging and age-related diseases such as Parkinson's Disease are in part due to decrease in expression of mitochondrial complex I subunits. By contrast, diminishing expression of mitochondrial complex I genes in lower organisms increases lifespan. Furthermore, metformin, a putative complex I inhibitor, is thought to increase healthspan in mammals. We investigated whether loss of one allele of *Ndufs2*, the catalytic subunit of mitochondrial complex, impact positively or negatively healthspan and lifespan in mice. Our results indicate that *Ndufs2* hemizygous mice from birth (*Ndufs*$^{+/-}$) show no overt impairment in motor function, learning, tissue histology, organismal metabolism or sensitivity to metformin in C57B6/J mice. However, there are detectable changes at the level of gene expression in individual cell types and tissues due to loss of one allele of *Ndufs2*. Our data indicate that decline in mitochondrial complex I subunit NDUFS2 is not detrimental to healthspan.

A hypomorphic mouse model identified Fis1 as a required protein for mitochondrial ultrastructure and function

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Mitochondrial fission is fundamental for a plethora of cellular processes. Fission protein 1 (Fis1) was identified in yeasts as the first receptor of Drp1, the master regulator of mitochondrial fission. Fis1 was later shown to be dispensable for the division of mitochondria in mammals1, where its role remains highly controversial and its relevance in mitochondrial physiology unexplored. To elucidate the physiological roles of Fis1 we generated a hypomorphic mouse model, characterised by a constitutive Fis1 downregulation, and a Fis1-floxed, FIS1flfl, mouse line. Fis1 hypomorphism resulted incompatible with life. Fis1 hypomorphic (FIS1hh) pups develop a severe phenotype that resembles a mitochondrial disease, becoming hypoactive and kyphotic within just two weeks of life, ultimately dying 12-18 days after birth. Mitochondrial ultrastructure is severely compromised in asymptomatic FIS1hh pups, as well as mitochondrial respiration. These Fis1hh defunct mitochondria elicit not only a mitochondrial integrative-stress response (ISRmt), with upregulation of Fgf21 and GDF15, but also the cGAS-STING pathway, consequently exacerbating inflammatory responses that deteriorate tissue homeostasis. Remarkably, depletion of Fis1 from adult life (using Fis1flfl mice) mimics the Fis1hh phenotype. We have shown that Fis1 is an essential mammalian protein and that its loss is incompatible with life.

A role for mitochondria in the regulation of cholesterol biosynthesis

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Mitofusin 2 (MFN2), an outer mitochondrial membrane GTPase that mediates mitochondrial fusion, has recently been shown to regulate phospholipid transfer between mitochondria and the endoplasmic reticulum (ER). Whether it plays a role in regulating other aspects of lipid metabolism is little understood. Here, we report that human, murine, and yeast cells deficient for *Mfn2/Fzo1* have higher levels of free cholesterol. We pinpointed this change in *Mfn2*$^{-/-}$ cells to an increased uptake of glutamine that promotes expression of HMGCR, the rate-limiting enzyme in cholesterol synthesis. Genetic ablation of the mitochondrial glutamine transporter in *Mfn2*$^{-/-}$ cells reverted HMGCR levels to that in wild-type cells. Remarkably, glutamine-deprivation and the small molecule-inhibition of mitochondrial glutamine oxidation in wild-type cells depleted HMGCR levels. Thus, our data reveal a previously unappreciated role for mitochondria in the regulation of cholesterol synthesis.

Peculiar post-transcriptional modification of *Chlamydomonas reinhardtii* mitochondrial mRNAs

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Mitochondria are semi-autonomous organelles holding a complete gene expression machinery. Since their bacterial origin, mitochondria have diverged during eukaryote evolution acquiring particular features in a specific phylae-manner (Kummer and Ban, 2021). Knowledge in mechanisms underlying mitochondrial gene expression is still missing, particularly in photosynthetic organisms.

Chlamydomonas reinhardtii, a unicellular green alga, represents an exciting model to study this process. Its mitochondrial genome is small and compact and its transcription generates two polycistronic transcripts, which are endonucleolytically cleaved into monocistronic mRNAs, tRNAs, and fragmented rRNAs (Boer and Gray, 1988). Analysis of the 8 mitochondrial mRNAs highlighted peculiar features (Salinas-Giegé et al., 2017). First, they do not have 5' untranslated region (UTR) and start at the AUG initiation codon. Second, mature mRNAs possess post-transcriptionally added C-rich tails at the 3' extremity of their 3'UTR. These C-rich tails are specific to mature mitochondrial mRNAs and found only in the *Chlorophyceae* group from which Chlamydomonas belong. However, their precise role remains unknown.

We currently identified two Nucleotidyltransferases that could potentially be involved in polycytidylation. Our analysis showed that these two enzymes are specific to *Chlorophyceae* class and imported into mitochondria. We also identified two Octotricopeptide repeat (OPR) proteins that may cleave the polycistronic transcripts thanks to their RNA-binding domain abundant in Apicomplexans (RAP domain) which have structural similarities with endonucleases (Boehm et al., 2017). Both are mitochondrial targeted. Biochemical and genetic studies are underway to determine which of the proteins are involved in *Chlamydomonas* mitochondrial mRNA modification and how they affect the mitochondrial genome expression.

Decreasing pdzd8-mediated mitochondrial-ER contacts in neurons improves fitness by increasing mitophagy

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The complex cellular architecture of neurons combined with their longevity makes maintaining a healthy mitochondrial network particularly important and challenging. One of the many roles of mitochondrial-ER contact sites (MERCs) is to mediate mitochondrial quality control through regulating mitochondrial turn over. Pdzd8 is a newly discovered MERC protein, the organismal functions of which have not yet been explored. Here we identify and provide the first functional characterization of the *Drosophila melanogaster* ortholog of Pdzd8. We find that reducing pdzd8-mediated MERCs in neurons slows age-associated decline in locomotor activity and increases lifespan in *Drosophila*. The protective effects of pdzd8 knockdown in neurons correlate with an increase in mitophagy, suggesting that increased mitochondrial turnover may support healthy aging of neurons. In contrast, increasing MERCs by expressing a constitutive, synthetic ER-mitochondria tether disrupts mitochondrial transport and synapse formation, accelerates age-related decline in locomotion and reduces lifespan. We also show that depletion of pdzd8 rescues the locomotor defects characterizing an Alzheimer's disease (AD) fly model over-expressing Amyloid-beta$_{1\text{-}42}$ ($A\beta_{42}$) and prolongs the survival of flies fed with mitochondrial toxins. Together, our results provide the first *in vivo* evidence that MERCs mediated by the tethering protein pdzd8 play a critical role in the regulation of mitochondrial quality control and neuronal homeostasis.

Direct interaction with DRP1 activates BAX and induces apoptosis

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The apoptosis executioner BAX and the dynamin-like protein DRP1 co-localize at mitochondria during apoptosis to mediate mitochondrial permeabilization and fragmentation. However, the molecular basis and functional consequences of their interplay remain unknown. Here, we show that BAX and DRP1 can physically interact and that their association is enhanced during apoptosis. We found that complex formation between BAX and DRP1 takes place exclusively in the membrane environment and involves several surfaces in BAX, of which the N-terminal region is required. Furthermore, the interaction of BAX with DRP1 is functionally relevant, since it modulates the activity of both proteins. Remarkably, when forced to dimerize, both BAX and DRP1 become activated and translocate to mitochondria, where they induce mitochondrial remodeling and permeabilization, resulting in apoptosis even in the absence of apoptotic triggers. Based on this, we propose that DRP1 can promote apoptosis by acting as a non-canonical direct activator of BAX through physical contacts with its N-terminal region.

Ultrastructure Expansion Microscopy in *Trypanosoma brucei*

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Trypanosoma brucei contains a single, tubular mitochondrial organelle with a uniquely organized mitochondrial DNA called kinetoplast DNA (kDNA). The kDNA consists of catenated mini- and maxicircles that together form a disk-like network which is physically linked to the basal body. The structure mediating the linkage is termed the tripartite attachment complex (TAC). The TAC spans three regions in the cell and currently more than 10 different proteins have been associated to that machinery. In order to assess the dynamics of TAC assembly I have established Ultrastructure expansion microscopy (U-ExM) that allows for isotropic expansion of T*. brucei* cells by a factor of four to five. By applying U-ExM we are able to overcome optical limitation of resolution problem in microscopy field. Here, I present results on the evaluation of U-ExM in trypanosomes and TAC component localization relative to each other and the kDNA.

Mutant mitochondrial DNA polymerase impairs acute antiviral immunity

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The roles of mitochondria, in the crossroads of growth, repair and energy metabolism, have recently emerged as important contributors of innate immunity. However, whether genetic mutations or variants affecting mitochondrial metabolism also contribute to susceptibility to infections is unknown. Here we show that mutant DNA polymerase gamma (POLG1), the nuclear-encoded mitochondrial DNA replicase, modulates antiviral innate immunity. We find that a common recessive *POLG1* patient mutation weakens innate immunity and modifies inflammatory responses in patient materials. A challenge with pathogen-associated molecular patterns mimicking viral exposure or actual viral infection resulted in dysregulated interferon-stimulated gene expression, promoting viral replication in mutant cells, followed by an augmented cytokine response. Our evidence suggests that viral infections can contribute to mitochondrial disease manifestations in patients with genetic predisposition, with major therapeutic implications for mitochondrial diseases.

Maintenance of proteostasis by mitochondrial stress response

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Mitochondria are organelles essential for energy production, cell signaling and cell death. Mitochondrial dysfunction and impaired proteostasis are common hallmarks of age-related diseases. Therefore, it is important to understand the interplay between mitochondria and cellular proteostasis. Mitochondrial dysfunction often causes mitochondrial protein import defects resulting in the accumulation of mitochondrial proteins in the cytosol. In addition, excessive reactive oxygen species may lead to oxidation of proteins and their misfolding. It was previously reported that proteasome activity is increased by mistargeted mitochondrial proteins in yeast. However, it is unclear whether similar mechanisms exist to remove unnecessary or damaged proteins in higher eukaryotes. Here, I will show how human cells respond to mitochondrial stress to maintain proteostasis.

Mechanisms of MDV biogenesis: MIRO1/2 and DRP1 mediate removal of assembled protein complexes

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Mitochondrial-derived vesicles (MDVs) are implicated in diverse physiological processes e.g. mitochondrial quality control and are linked to various neurodegenerative diseases. However, their specific cargo composition and complex molecular biogenesis is still unknown. Here we report the first proteome and lipidome of steady-state TOMM20^{+}-MDVs. We identified 106 novel high confidence MDV cargoes including all b-barrel proteins and the TOM import complex. MDV cargoes are delivered as fully assembled complexes to lysosomes, thus representing a novel mitochondrial quality control mechanism for multisubunit complexes including the TOM machinery. Moreover, we define key biogenesis steps of phosphatidic acid enriched MDVs starting with the MIRO1/2-dependent formation of thin membrane protrusions pulled along microtubule filaments, followed by MID49/MID51/MFF-dependent recruitment of the dynamin family GTPase DRP1 and finally DRP1-dependent scission. In sum, we define the function of MDVs in mitochondrial quality control and present the first mechanistic model for global GTPase-driven MDV biogenesis.

The Proteasome: Friend or Foe of Mitochondrial Protein Import?

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Most mitochondrial proteins are synthesized into the cytosol and subsequently translocated to mitochondria. A functional mitochondrial protein import is essential for cellular fitness and survival. Defects in mitochondrial protein translocation are associated with oxidative stress, neurodegenerative diseases as well as metabolic disorders. Mitochondrial import defects trigger a tightly regulated stress response and arrest cell division. In yeast, mitochondrial precursor proteins in the cytosol induce the ubiquitin-proteasome system via the transcription factor Rpn4 and thereby stimulates the degradation of cytosolic precursors. Surprisingly, abolishing the Rpn4-dependent proteasome response partly suppresses the precursor-induced growth arrest. If cells are unable to upregulate the proteasome, they trigger an alternative program and induce a specific subset of the cytosolic chaperone system. Apparently, the proteasome competes with the import system for mitochondrial precursors. Our data are compatible with the idea that these factors allow the accumulation of precursors in the cytosol in specific deposits, from where they can be re-routed onto a productive import pathway, once the import block is resolved. Obviously, cells can utilize different programs to mitigate the toxicity of mitochondrial precursor proteins.

The modified mitochondrial outer membrane carrier MTCH2 regulates mitochondrial fusion in response to lipogenesis

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Mitochondrial function is integrated with cellular status through the regulation of opposing mitochondrial fusion and division events. Here we uncover a link between mitochondrial dynamics and lipid metabolism by examining the cellular role of mitochondrial carrier homologue 2 (MTCH2). MTCH2 is a modified outer mitochondrial membrane carrier protein implicated in the regulation of fatty acid metabolism and intrinsic cell death. Our data indicate that MTCH2 is a selective essential effector of starvation-induced mitochondrial hyperfusion, a cytoprotective response to nutrient deprivation. We find that MTCH2 stimulates mitochondrial fusion in a manner dependent on the bioactive lipogenesis intermediate lysophosphatidic acid. We propose that MTCH2 monitors flux through the de novo lipogenesis pathway and transmits this information to the mitochondrial fusion machinery to promote mitochondrial elongation, enhanced energy production, and cellular survival under homeostatic and starvation conditions. These findings will help resolve the roles of both MTCH2 and mitochondria in tissue-specific lipid metabolism in animals.

Lipid transport to mitochondria during plant adaptation to phosphate starvation

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Phosphate (Pi) starvation is a frequent nutrient stress encountered by plants to which they adapt by exerting different mechanisms. The partial degradation of phospholipids is a widespread response observed in plants to increase the intracellular Pi availability. To maintain membrane integrity, the degraded phospholipids are replaced by a non-phosphorous plastid-synthetized lipid, the digalactosyldiacylglycerol (DGDG). This replacement implies lipid transport from plastids to other organelles. DGDG transport to mitochondria under Pi stress is thought to happen by non-vesicular lipid transport. Recently, we identified the mitochondrial transmembrane lipoprotein (MTL) complex, a huge complex enriched in lipids and mainly composed of proteins located in both mitochondrial membranes. One of its components of the inner membrane, AtMic60, was shown to play a role in intra-mitochondrial DGDG trafficking. To better understand the organization, structure and partners of the MTL complex, we are currently optimizing two approaches: 1) the purification of the complex in presence or absence of Pi for proteomic and structural analyses by cryo-EM and 2) co-immunoprecipitation (co-IPs) experiments using AtTom40 as bait. Here, we show the most promising purification results and the first cryo-electron microscopy images of the MTL complex. We also present our investigations on a putative plant Vps13 homolog identified by co-IP, a lipid transfer protein located at MCSs in yeast and mammals. We are currently investigating its subcellular localization and its role in mitochondrial lipid homeostasis. The new insights gained during this work will allow us to better understand the mechanisms implied in lipid transport to mitochondria in plants.

Homo-dimerization of the mitochondrial complex IV and its role in muscle biology and regeneration

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According to the plasticity model, the mitochondrial electron transport chain is a dynamic system in which monomeric respiratory complexes can be physically linked together in larger structures called super-complexes in order to optimize its function according to metabolic cell demands. The homo and hetero super-assembly of CIV have been proposed to mediated by a family of proteins of complex IV, the Cox7a family which is composed by three members. Cox7a1 would mediate the homo dimerization of CIV, Cox7a2 would stabilize the monomer form and Cox7a2l mediates the super-assembly between complex III and complex IV (Cogliati et al. Nature 2016). In this project we focused on the role of Cox7a1. Cox7a1 is highly expressed in skeletal and heart muscle. Moreover, it has been proposed as an embryonic-transition marker given that its expression starts right after mammals' birth (West et al. Oncotarget 2017). Using zebrafish and mouse genetic models we have probed its role in the homo-dimerization and homo-multimerization of complex IV. Zebrafish null allele models for *cox7a1* resulted to have reduced skeletal muscle mass with consequences in their exercise capacity but, however, they also showed an improvement in their heart regeneration capacity. Evidences suggest that the homo dimerization of complex IV mediated by Cox7a1 confers a metabolic maturation to the muscle tissue with negative consequences for the regenerative capacity of the heart but positive for proper muscle strength. This work present Cox7a1 as a possible target to improve the regenerative capacity of the heart.

Human mitochondrial ferredoxins 1 and 2 perform different biosynthetic functions

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Ferredoxins (FDXs) constitute a family of redox proteins found in all kingdoms of life, their iron sulfur (Fe/S) cofactor enabling them to transfer single electrons in numerous biological pathways. Two subclasses of [2Fe-2S] ferredoxins, represented by, e.g., mitochondrial FDX2 and FDX1 (aka adrenodoxin), are essential for cellular Fe/S protein biogenesis and cytochrome P450 steroid transformations, respectively. While FDX1's P450 function is well-established, an additional involvement in Fe/S protein biogenesis is controversial. Here, we resolved this open question, and we identified novel physiological functions of the two human ferredoxins. We show that FDX2, but not FDX1, serves as the specific electron donor for Fe/S protein maturation. Vice versa, FDX1 is specifically required for heme *a* and lipoyl cofactor biosynthesis. In the latter pathway, FDX1 feeds electrons into the radical chain reaction of lipoyl synthase. This enzyme was identified as the target of the copper toxicity of elesclomol, an anti-tumor drug. The striking target specificity of both ferredoxins despite their almost identical folding can be explained by different surface potentials and short conserved sequence motifs in each ferredoxin. Swapping these motifs partially reversed the target specificity of these crucial electron donors. Overall, FDX1 and 2 fulfill diverse but highly specific functions in human mitochondria.

Structural heterogeneity of the respiratory chain ensures optimal OXPHOS performance

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The structural organization of the mitochondrial respiratory chain (MRC) has been intensely debated for over 60 years. The mammalian MRC is formed by complexes I, III and IV that can associate in supercomplexes (SCs) and respirasomes, whose biogenetic regulation and functional properties remain unclear. We have addressed the relevance of the structural and functional heterogeneity of the human MRC. In our work, we show the coexistence of two separated MRC organizations, defined by the differential expression of COX7A subunit isoforms, namely COX7A2 or COX7A2L/SCAFI. Each COX7A isoform promotes effective functional reorganization of co-existing MRC structures, thus preventing MRC deficiency. Notably, SCAFI-dependent MRC reorganization is reversibly regulated by COX7A2 levels, and it is unable to fully compensate for the absence of COX7A2 in terms of bioenergetics efficiency. This supports the idea that COX7A2 is the primary structural “organizer” of the human MRC, with SCAFI taking over as a rescue mechanism aimed to maintain mitochondrial function. We conclude that its structural heterogeneity guarantees optimal MRC function.

Leveraging Mass Spectrometry to Uncover Mitochondrial Adaptations to Hypoxia Exposure

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Synthesis of molecules like ATP, nucleotides, and lipids require direct input of electrons into the mitochondrial electron transport chain (ETC). In order to re-oxidize ubiquinol (QH2) and maintain synthesis of these molecules, electrons are deposited on a terminal electron acceptor, known to be oxygen (O_2) in mammals. Paradoxically, we find that electron input into the ETC is sustained in cells lacking O2 reduction, suggesting the existence of another terminal electron acceptor. In this study we leverage mass spectrometry to uncover a secondary terminal electron acceptor in the mammalian ETC. Upon exposure to hypoxia, mammalian cells exploit this adaptive electron acceptor to maintain mitochondrial functions dependent on electron flow in the ETC such as pyrimidine biosynthesis. The capacity to utilize this adaptive terminal electron acceptor is tissue-specific, suggesting the presence of multi-tiered regulatory mechanisms on this pathway. Thus, we delineate a previously unappreciated circuit of electron flow in the mammalian ETC that maintains mitochondrial function under O2 limitation in vitro and in vivo.

Mapping the substrate binding site of the mitochondrial ADP/ATP carrier

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Mitochondrial ADP/ATP carriers provide key transport steps in eukaryotic oxidative phosphorylation by importing ADP into the mitochondrial matrix and by exporting synthesized ATP to fuel cellular processes. Structures of the inhibited cytoplasmic- and matrix-open states have confirmed an alternating access mechanism, but the molecular nature of substrate binding remains unresolved. After investigating all solvent-exposed residues in the translocation pathway in both conformational states, we identified a single substrate binding site in the center of the cavity. It consists of three positively charged residues, which could bind the phosphate groups of the adenine nucleotides and a cluster of three aliphatic and one aromatic residue, which together could bind the adenosine moiety. In addition, two arginine/asparagine pairs on either side of the main site are found to play an important role, potentially for positioning, ensuring strict specificity for adenine di- and tri-nucleotides. Importantly, the same residues are involved in binding of both ADP and ATP, indicating that the import and export steps occur consecutively and in a reversible way. The features of the identified binding site explain the electrogenic nature of transport, providing answers on the bioenergetics of adenine nucleotide transport in mitochondria.

Mitochondrial T cell dysfunction accelerates intestinal ageing

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Demographic ageing is becoming a major burden due to the increase in multimorbidity and its age-related diseases. Therefore, the design of new strategies to foster healthier ageing is currently urging. The intestine is a physical and immunological barrier that harbours a microbial ecosystem termed gut microbiota, and whose age-associated deterioration could unleash the development of such pathologies. Specifically, host-microbiota symbiosis is lost during ageing due to aberrant germinal centre (GC) responses that support perturbations in gut microbial communities, driving gut dysbiosis. Along with a pro-inflammatory environment, gut dysbiosis enhances intestinal permeability favouring the systemic dissemination of bacterial products. This event contributes to an age-related chronic inflammation (inflammageing), which is linked to cardiometabolic and neurologic disorders associated to ageing. Previous data from the laboratory demonstrate that *Cd4*^{Cre}*Tfam*$^{fl/fl}$ mice, carrying a mitochondrial dysfunction in T cells, display inflammageing that results in multimorbidity (i.e., cardiovascular alterations and cognitive dysfunction). Thus, we hypothesised whether T cell metabolic failure leads to the loss of intestinal homeostasis triggering premature ageing in this mouse model. Our findings indicate that young *Cd4*^{Cre}*Tfam*$^{fl/fl}$ mice show altered intestinal T cell populations with increased markers of activation and senescence, as well as dysregulated GC responses compared to control mice. Accordingly, the analysis of *Cd4*^{Cre}*Tfam*$^{fl/fl}$ mouse faecal bacteria reveals a disrupted configuration of the gut microbiota. Finally, *in vivo* experiments support a premature alteration in the physical barrier function. Overall, our results suggest that mitochondrial T cell dysfunction perturbs intestinal T cell subsets, affecting host-microbiome symbiosis and intestinal barrier integrity.

Mitochondrial OXPHOS regulates dietary lipid transport in enterocytes

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Patients with mutations affecting mitochondrial function often present with gastrointestinal symptoms associated with malnutrition, however, the function of mitochondria in the intestine remains poorly studied. The intestinal epithelium performs important functions in nutrient absorption and metabolic regulation, and forms a structural barrier between the luminal microbiota and mucosal immune cells. Enterocytes, the absorptive cells of the intestine, facilitate the efficient uptake, mobilization and transport of dietary nutrients, including fats. Digested lipids taken up by enterocytes are re-esterified and assembled into triglyceride-rich lipoproteins primarily in the form of chylomicrons (CMs), which are then released in the circulation to supply peripheral organs with lipids. The function of mitochondria in intestinal lipid trafficking and secretion remains unknown. To study the role of mitochondria in the intestine, we generated mice with intestinal epithelial cell (IEC)-specific ablation of DARS2, a mitochondrial-specific aspartyl - tRNA synthetase that is essential for mitochondrial translation of the 13 mtDNA - encoded OXPHOS subunits. IEC-specific DARS2 deficiency caused severe loss of body weight and failure to thrive. Histological examination of intestinal tissue from these mice revealed massive accumulation of lipids within large lipid droplets (LDs) in enterocytes of the proximal small intestine that is responsible for nutrient absorption. Feeding with a fat-free diet prevented fat accumulation in LDs in enterocytes of $DARS2^{IEC\text{-}KO}$ mice, showing that these contain primarily dietary lipids. In addition, postprandial studies revealed that DARS2 deficiency in IECs impaired dietary lipid transport to the plasma and peripheral organs. Furthermore, TEM revealed a distinct lack of mature chylomicrons in DARS2-deficient enterocytes, suggesting that DARS2-deficiency inhibits chylomicron formation. IEC specific ablation of SDHA and COX10, which are essential subunits of respiratory complexes II and IV respectively, caused a similar phenotype to DARS2 deficiency, demonstrating that mitochondrial dysfunction causes defective lipid transport and lipid accumulation in enterocytes. Our results reveal an essential function of mitochondria in regulating chylomicron production and lipid transport in enterocytes, a mechanism that is relevant for understanding the intestinal and nutritional defects observed in patients with mitochondrial defects.

Sub-lethal apoptotic stress enables mtDNA release and drives the senescence-associated secretory phenotype

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Cellular senescence is a stress response implicated in ageing and age-related diseases. Senescent cells can secrete pro-inflammatory factors, which are collectively known as the senescence-associated secretory phenotype (SASP). Importantly, mitochondria were shown to regulate the senescence-associated secretory phenotype (SASP) (Correia-Melo et al., 2016). However, the exact mechanisms via which mitochondria contribute to the SASP remain to be elucidated. In this study, we discovered that senescent cells undergo sub-lethal apoptotic stress, consisting of the activation of pro-apoptotic BAK and BAX, and the release of cytochrome c and mtDNA into the cytosol. BAK and BAX were found to mediate the leakage of mtDNA in senescent cells and regulate the SASP.

Global kinome profiling reveals DYRK1A as critical activator of the human mitochondrial import machinery

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Mitochondria require an extensive and functional proteome to maintain a plethora of metabolic reactions and changes in cellular demands depend on a rapid adaptation of the mitochondrial protein composition. The translocase of the outer membrane TOM constitutes the organellar entry gate that imports nearly all proteins as precursors from the cytosol. Therefore, TOM presents the ideal target to adjust the mitochondrial proteome by regulating the organellar protein influx. Here, we perform a global in vitro profiling of the human kinome and identify that the cytosolic kinase DYRK1A phosphorylates the mitochondrial import receptor TOM70 exclusively at position Ser91. Inhibition of DYRK1A, which has not been linked to mitochondria before, impacts on mitochondrial structure and function in vivo and elicits a protective transcriptional response to maintain a functional import machinery. On the molecular level phosphorylation of TOM70Ser91 by DYRK1A stimulates the interaction of the typically peripheral import receptor with the core TOM complex. This association enables the transfer of receptor-bound precursors to the translocation pore and initiates their import. Consequently, loss of TOM70Ser91 phosphorylation results in a strong decrease in import capacity of metabolite carriers. Therefore, DYRK1A plays a critical role in the activation and maintenance of the carrier import pathway in human mitochondria. The identification of this novel link between DYRK1A and mitochondria will allow new insights into disease mechanisms caused by dysfunctional DYRK1A, including autism spectrum disorder, microcephaly, and Down syndrome.

The impact of age-associated mitochondrial alterations in intestinal tumour development

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During ageing the clonal expansion of mitochondrial DNA (mtDNA) point mutations in colonic epithelial crypts, leads to defects in mitochondrial oxidative dysfunction (OXPHOS). In addition, OXPHOS dysfunction has been shown to be caused by mtDNA mutations in human colorectal adenocarcinomas. However, what impact age-related mtDNA mutations have on tumorigenesis has been a topic of controversy. To directly address this question, we crossed the progeroid mtDNA mutator mouse model (*PolgA*$^{mut/mut}$), harbouring an accelerated accumulation of mtDNA mutations with age, with an inducible mouse model of intestinal adenoma development (*Lgr5-creER/Apc*$^{fl/fl}$). Resulting *PolgA*$^{mut/mut}$*/Lgr5-creER/Apc*$^{fl/fl}$ mice display a shorter life expectancy due to accelerated tumour growth, compared with control mice. At the gene and protein level we show in *PolgA*$^{mut/mut}$ mice an upregulation of the *de novo* serine synthesis pathway (SSP) and serine-uptake and metabolism associated with age. At a functional level using *ex vivo* cultures of intestinal adenoma organoids, we confirm a reduced oxidative respiratory capacity and upregulation of *de novo* serine synthesis in *PolgA*$^{mut/mut}$*/Lgr5-creER/Apc*$^{fl/fl}$ mice, which additionally displayed a resilience to serine and glycine starvation compared to controls. In corroboration, we show the ageing human intestine and colorectal cancer biopsies to display age-related mitochondrial dysfunction and associated metabolic remodelling with an enrichment of clonally expanded mtDNA mutations and related OXPHOS dysfunction through the adenocarcinoma sequence. These data demonstrate in response to OXPHOS dysfunction with age, normal intestinal crypts undergo metabolic remodelling, which provides substrates for anabolic and antioxidant pathways, and thus a selective advantage for tumour growth and survival.

Co-Deletion of ATAD1 with PTEN primes cells for BIM-mediated apoptosis

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One of the most common genetic aberrations in human cancer is a deletion on chromosome 10q23 that encompasses PTEN, a potent tumor suppressor gene. Such deletions frequently include “innocent bystander” loci that are adjacent to PTEN. Understanding how the loss of these neighboring loci affects the biology of the cancer cell could identify therapeutic targets for precision oncology. Here, we discover that loss of ATAD1, which is adjacent to PTEN, increases apoptotic priming and renders cancer cells vulnerable to proteotoxic stress. ATAD1 acts by directly and specifically extracting the pro-apoptotic BIM protein from mitochondria. Thus, we report the discovery of a new negative apoptotic regulator that modulates the BCL2 family with clinical implications in cancer and beyond.