Contents

Poster

P1 - Mitochondrial impairment precedes lysosomal deficiency in a Parkinson's disease model of LRRK2 toxicity

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Deciphering the molecular principles governing Parkinson's disease (PD) is a yet incompletely accomplished task of modern medicine. Mutations in the gene coding for the leucine-rich repeat kinase 2 (LRRK2) are the most prevalent cause of familial PD. Although accumulating evidence suggests that this protein affects mitochondrial and lysosomal function, the molecular regimes that govern LRRK2-driven cell death, as well as the chronological order of these pathophysiological events, remain enigmatic.

In this work, we established a humanized yeast model for PD, heterologously expressing LRRK2, and studied its toxicity during ageing. Early after start of its expression, we observed mitochondrial localization of LRRK2, accompanied by a selective misassembly of the respiratory chain complex IV and a downregulation of mitochondria-related transcripts. With increasing age, this resulted in pronounced reduction of mitochondrial mass despite the absence of excessive mitochondrial degradation, suggesting an inhibition of mitochondrial biogenesis by LRRK2. Further, lysosomal degradative pathways, including autophagy and mutlivesicular body pathway were dysregulated by LRRK2 in old cells, subsequently resulting in age-depended cell death.

In sum, these results provide novel perspectives of LRRK2-associated pathogenesis during PD, suggesting mitochondrial impairment as a primary event of LRRK2-caused cytotoxicity, followed by an inhibition of lysosomal degradative capacity and cell death.

P2 - Levels of TFAM expression affect mitochondrial function in vivo

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Mitochondrial transcription factor A (TFAM) has emerged as an essential regulator of mitochondrial function in mammals due to its dual role as a core component of the mitochondrial transcription machinery and a key packaging factor of the mitochondrial genome. Moreover, *in vitro* studies have pointed out that varying TFAM-to-mtDNA ratios serve as an important mechanism for control of mtDNA maintenance and expression. To further decipher the effect of TFAM levels on mitochondrial gene expression *in vivo*, we generated transgenic mice expressing mouse TFAM at different levels (BAC-TFAM mice). Differences in mtDNA copy number, mitochondrial transcripts and respiratory chain components in relation to net TFAM expression were assessed. BAC transgenic mice expressing TFAM at increased levels (within a likely normal physiological range) are healthy and indistinguishable from controls. In contrast, mice strongly overexpressing TFAM display a general growth defect and die at an early age, arguing for the necessity to carefully titrate TFAM-to-mtDNA ratios *in vivo*. As mild TFAM overexpression and a concomitant mtDNA increase was recently shown to rescue disease manifestations of the mtDNA mutator phenotype, potential physiological benefits of TFAM overexpression as such will be addressed after exercise challenges *in vivo*.

P3 - Reconstituting mitochondrial translation in vitro

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Bacterial translation has been extensively studied over the last decades, but the evolutionarily related mitochondrial translation machinery is to date poorly investigated. It is not until in the last few years that structures of mitochondrial ribosomes and some of their interaction partners have become available, but functional biochemical studies are still missing. The field of research on mitochondrial gene expression is hampered by the lack of an *in vitro* translation system, an important tool for confirming and further elaborating observations made *in vivo* and for detailed mechanistic studies of the individual steps of the translation process.

Our primary goal is to set up a yeast mitochondrial *in vitro* translation system, based on individually purified components, with ribosomes that can be initiated with native mitochondrial mRNAs and that are active in peptide bond formation. Even such a minimalistic system can be used to study fundamental aspects of yeast mitochondrial translation, for example the rate and accuracy of tRNA selection in the ribosomal A site. From this starting point the *in vitro* system can be expanded to include a more complete set of translation factors and applied to study other parts of the mitochondrial translation process.

P4 - Characterization of GTP-binding proteins and their role in mitochondrial ribosome assembly

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Mitochondria are essential organelles as they represent the primary energy producers within eukaryotic cells. They have their own DNA which encodes for hydrophobic constituents of the oxidative phosphorylation system (OXPHOS). Mitoribosomes are ribonucleoprotein complexes structurally and functionally conserved across vertebrates that are responsible for the catalysis of protein synthesis. The mechanisms of assembly of the mitoribosome and the factors involved in this process still need to be characterised. In bacteria, GTPases belonging to the Obg family are known to interact with the large ribosomal subunit (LSU). In this study we investigate the role of two human ObgE homologues, GTPBP10 and GTPBP5. We showed that these proteins localise to mitochondria and interact specifically with the mtLSU and, in the presence of GTP analogue, they exhibit a different mechanism of action. We also show that depletion of GTPBP10 or GTPBP5 leads to impairment in the OXPHOS system, general reduction in mitochondrial translation and decreased monosome levels. Preliminary results from cryo-electron microscopy analysis of GTPBP10-mtLSU interaction show the binding site of this protein to the mtLSU, giving further insight into the function of this protein. Overall, our data suggest the importance of these GTPases in the late stage of mtLSU assembly.

P5 - First Recessive Variant In HECW2 Gene Causes Neurodevelopmental Disorder With Hypotonia, Seizures, And Absent Language (NDHSAL))

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The E3 ubiquitin-protein ligase HECW2 (also known as NEDL2) has been linked to neurodevelopmental disorder with hypotonia, seizures, and absent language (NDHSAL; MIM:617268). Currently, only 10 patients with 4 different *de novo* variants have been described in *HECW2* gene (MIM:617245) related to NDHSAL. We report the clinical, biochemical and genetic aspects of a Caucasian girl who presents NDHSAL with neonatal-onset epilepsy, and deficiency of the complex IV of the respiratory chain and mitochondrial DNA depletion. Whole-Exome Sequencing (WES) uncovered a novel variant in homozygosis (NC_000002.11: g.197092813_197092824delinsAC) in the *HECW2* gene (NM_001348768.2; c.3720_3872del; p.Leu1256_Trp1306del51). This is the first patient with NDHSAL caused by an inherited recessive variant in *HECW2* gene. The "*in silico*" study predicted the loss of exon 22 in the transcript and its consequent change in the structure of the protein domain HECT, key for the interaction with the target proteins. Sequencing of the *HECW2* cDNA revealed a smaller size and the loss of exon 22. Furthermore, a decreases levels in the protein respect to the controls was showed.

Previous studies have demonstrated that HECW2 can play a key role in neurological development and in the maintenance of the OxPhos system and mitochondrial function. More detailed studies are needed to clarify the mechanisms involved.

P6 - Analysis of pathogenic mtDNA mutations in Serbian patients with MELAS

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Background: Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), considered one of multisystem diseases inherited by mitochondrial DNA (mtDNA) in which the mutated or impaired mitochondria implicated in a defective mitochondrial protein synthesis and reduced activities of respiratory chain complexes. Furthermore, energetic impairment and accentuated free radical generation are caused by mitochondrial oxidative phosphorylation pathway defect.

Objective: The aim of this study was to perform mutational screening of entire mtDNA in Serbian families clinically diagnosed with MELAS.

Material and Methods: Individuals included in this study were recruited from the child and adult neurology hospitals in Belgrade, Serbia. All examined individuals had characteristic clinical presentation suggesting the presence of MELAS. Molecular genetic analysis of entire mtDNA was performed by Sanger sequencing.

Results: We have detected heteroplasmic mt.3243 A>G mutation in MT-TL1 gene in 2 Serbian families. The A>G transition of the mitochondrial nucleotide pair 3243 in the dihydrouridine loop of mitochondrial tRNALeu (UUR) is specific to patients with MELAS. Number of other variants were detected in various parts of mitochiondrial genome in both families.

Conclusion: Understanding of the genetic background of mitochondriopathies may further facilitate the diagnostic approach and open perspectives to future, possibly therapies.

P7 - SSBP1 mutations cause a complex optic atrophy spectrum disorder with mitochondrial DNA depletion

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Inherited optic neuropathies include complex phenotypes, mostly driven by mitochondrial dysfunction. We report an optic atrophy spectrum disorder in adults, including retinal macular dystrophy and kidney insufficiency leading to transplantation, associated with mitochondrial DNA (mtDNA) depletion without accumulation of multiple deletions. By whole-exome sequencing, we identified mutations affecting the mitochondrial single strand binding protein (SSBP1) in four families with dominant and one with recessive inheritance. We show that SSBP1 mutations in patient-derived fibroblasts variably affect its amount and alter multimer formation, but not the binding to ssDNA. SSBP1 mutations impaired mtDNA, nucleoids and 7S-DNA amounts as well as mtDNA replication, impacting replisome machinery. The variable degrees of mtDNA depletion in cells is reflected in severity of mitochondrial dysfunction, including respiratory efficiency, OXPHOS subunits and complexes amount and assembly. mtDNA depletion and cytochrome c oxidase-negative cells were found ex vivo in biopsies of affected tissues, like kidney and skeletal muscle. Reduced efficiency of mtDNA replication was also reproduced in vitro, confirming the pathogenic mechanism. Furthermore, ssbp1 suppression in zebrafish reduced optic nerve size, a phenotype complemented by wild-type mRNA but not by SSBP1 mutant transcripts. This previously unrecognized disease of mtDNA maintenance implicates SSBP1 mutations as novel cause of human pathology.

P8 - Searching for the causes of cerebellar ataxia in mitochondrial diseases: insights from the Harlequin mouse.

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Mitochondrial diseases (MD) are a group of genetic disorders caused by dysfunction of oxidative phosphorylation system. One of the most sensitive organs to MD is nervous tissue, and accordingly, one of most prevalent manifestation of MD is neurodegeneration that frequently leads to cerebellar ataxia.

Our aim was to go in depth in the main causes of cerebellar ataxia in a mouse model of MD, the Harlequin mouse (Hq), and assess if an exercise-training program could exert any neuroprotective effect in this model. Our results demonstrated alterations in several proteins involved in synaptic plasticity and calcium homeostasis in the cerebellum of Hq mouse, that could not be counteracted by exercise-training despite an improvement in the physical performance of the exercise-trained Hq animals. Most of these proteins, such as glutamate transporters mGluR1 and GluRδ2, Homer-3 adaptor protein, protein kinase C (PKC), among others, have proven to be essential in the mechanism of synaptic plasticity named long-term depression (LTD), which seems to be dysfunctional in this model. These disturbances identified could be the start point of cerebellar ataxia in Hq mouse, opening new promising therapeutic targets in the context of MD-induced ataxia, results that could might be further extended to other neurodegenerative disorders.

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P9 - Complex III is essential for complete assembly and maturation of complex I in human mitochondria

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Mutations in the mitochondrial DNA (mtDNA) gene encoding complex III ($cIII_2$) subunit cytochrome b (*MT-CYB*) cause $cIII_2$-associated mitochondrial diseases of variable severity, depending of the nature of the mutation and the heteroplasmy levels. Severe *MT-CYB* mutations destabilizing the protein are commonly associated with complex I (cI) defects. We have performed thorough proteomic and biochemical analyses of a human cell line carrying a pathogenic 4-bp deletion in *MT-CYB*. Our results demonstrate that the concomitant cI deficiency originates from severe stalling of its assembly, leading to the accumulation of the late intermediate lacking the NADH-binding module, contrary to the previous proposal that the cI defect was due to post-assembly destabilization of the fully assembled holoenzyme. Additionally, we found a milder but significant cIV deficiency. These observations highlight the central role of $cIII_2$ for the overall biogenesis of the respiratory chain, particularly of cI, the complete maturation of which preferentially occurs within the 'respirasome' structures.

P10 - Loss of Mitofusin 2 in adult dopaminergic neurons causes a premature activation of surrounding microglial cells.

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Mitochondria are highly dynamic organelles requiring a tight balance between mitochondrial fusion and fission. Alterations of mitochondrial dynamics can compromise the function of mitochondrial network resulting in severe pathological conditions. Parkinson's disease is a common neurodegenerative disorder characterized by the preferential loss of dopaminergic (DA) neurons in the midbrain. Several lines of evidence indicate that mitochondrial dysfunction plays an essential role in DA cell death, although the underlying molecular mechanisms are still unclear. To investigate the role of mitochondrial dynamics in the nigrostriatal system, we generated mice lacking *Mitofusin 2* (*Mfn2*), a key player of mitochondrial fusion, in adult DA neurons. These mice developed a progressive phenotype with impaired spontaneous locomotion and profound DA degeneration. In these neurons, the loss of *Mfn2* leads to the disruption of the mitochondrial morphology followed by impaired respiratory chain function and mitochondrial DNA depletion. Transcriptomic analysis revealed that defective mitochondrial fusion in mature DA neurons causes a premature immune response. Unexpectedly, Mfn2 KO mice show an early activation of the surrounding microglial cells that largely precedes the degenerative process and might contribute or exacerbate the neuronal cell loss.

P11 - Using the TFAM-FLAG mice to study the modulation of mtDNA expression in vivo.

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Mitochondria contains their own genome (mtDNA) encoding 13 proteins essential for the function of the oxidative phosphorylation system (OXPHOS). In mammals, the mtDNA is compacted into dynamic structures called nucleoids, and the mitochondrial transcription factor A (TFAM) is the main packaging protein. The TFAM-to-mtDNA ratio is an important mechanism to regulate mtDNA copy number and expression. However, how this ratio is maintained is poorly understood. To gain insights into this regulatory process, we generated a TFAM-FLAG mouse model to investigate by mass spectrometry how the nucleoid composition affects mtDNA expression *in vivo*. TFAM-FLAG rescue mice born at Mendelian ratios and had no apparent phenotype. In heart tissue, the level of TFAM-FLAG protein was similar to TFAM in WT animals, and we detected normal levels of mtDNA content, mitochondrial transcripts, and OXPHOS proteins. In liver tissue, we found a small decrease of TFAM-FLAG protein, mtDNA content, and transcripts of light strand promoter, however, OXPHOS protein levels remain stable. Immunoprecipitation of TFAM-FLAG protein from liver mitochondria allowed us to purify mitochondrial nucleoids. Preliminary mass spectrometry data identified expected nucleoid interacting proteins such as the DNA polymerase gamma. Further improvements of mitochondrial nucleoid purification are required for comparisons among tissues.

P12 - Novel variants in genes of the mitoribosomal large subunit cause disorders of mitochondrial translation

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To date, only 13 of the ~80 mitoribosomal genes have been implicated in mitochondrial disease. Often presenting in infancy or early childhood, these mitoribosomal defects are a rare but expanding group of mitochondrial disease genes. We used whole exome sequencing (WES) in three unrelated patients presenting in childhood, with similar clinical symptoms typical of mitochondrial disease, identifying a homozygous (c.[646C>T], p.[Arg216*]) variant in MRPL47 in each case. A complete loss of steady-state MRPL47 protein is observed in the fibroblasts of patients harbouring this mutation, along with combined OXPHOS deficiency affecting complexes I and IV at both the steady-state and enzymatic level. Investigation of mitoribosome assembly reveals some loss of fully assembled large subunit affecting proteins with late-stage assembly. Analysis of mitochondrial translation products in fibroblasts demonstrates a generalised reduction in de novo mitochondrial protein synthesis.
WES of two further unrelated patients of Lebanese descent, presenting at the ages of two weeks and one year of age respectively, with a primarily muscle based phenotype typical of mitochondrial disease, identified compound heterozygous variants (c. [382G>T], p.[Glu128*] and c.[601+5G>A], p.[?]) and a homozygous c.[601+5G>A], p.[?] variant in the MRPL65 gene. In U2OS cells, the knock-down of MRPL65 results in a severe combined OXPHOS defect. In patient fibroblasts with the homozygous splice variant there is no loss of MRPL65 protein, however a significant amount of steadystate MRPL65 does not become incorporated in the fully assembled LSU. The variant affects the donor site of exon 1 causing intron retention in ~25% of transcripts in lymphocytes, though this may be higher in more affected tissues. These data demonstrate the functional effect of these mutations on mitochondrial translation and OXPHOS, establishing pathogenicity of variants in two novel disease genes.

P13 - C6orf203 is an RNA-binding protein involved in mitochondrial protein synthesis

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In all biological systems, RNAs are associated with RNA-binding proteins (RBPs), forming complexes that control gene regulatory mechanisms, from RNA synthesis to decay. In mammalian mitochondria, post-transcriptional regulation of gene expression is conducted by mitochondrial RBPs (mtRBPs) at various stages of mt-RNA metabolism, including polycistronic transcript production, its processing into individual transcripts, mt-RNA modifications, stability, translation, and degradation. To date, only a handful of mt-RBPs have been characterized. Here, we describe a putative human mitochondrial protein, C6orf203, that contains an S4-like domain - an evolutionarily conserved RNA-binding domain previously identified in proteins involved in translation. Our data show C6orf203 to bind highlystructured RNA in vitro and associate with the mitoribosomal large subunit in HEK293T cells. Knockout of C6orf203 leads to a decrease in mitochondrial translation and consequent OXPHOS deficiency, without affecting mitochondrial RNA levels. Although mitoribosome stability is not affected in C6orf203-depleted cells, mitoribosome profiling analysis revealed a global disruption of the association of mt-mRNAs with the mitoribosome, suggesting that C6orf203 may be required for the proper maturation and functioning of the mitoribosome. We therefore propose C6orf203 to be a novel RNA-binding protein involved in mitochondrial translation, expanding the repertoire of factors engaged in this process.

P14 - Deep sequencing of mitochondrial DNA and functional characterization of a novel POLG mutation in a patient with autosomal recessive progressive external ophthalmoplegia

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Progressive external ophthalmoplegia (PEO) can be caused by mutations in the *POLG* gene, encoding the alpha subunit of polymerase gamma (polγ). Polγ consists of the catalytic subunit, POLγA, containing polymerase- and exonuclease-activities and a dimer of POLγB (POLG2). This trimeric protein is a DNA polymerase and is essential for replication of mitochondrial DNA (mtDNA).

We describe a 69-year-old male patient who at age 55 noted slowly progressive bilateral ptosis and limitation in both horizontal and vertical gaze. Since the age of 65 he has had complete horizontal opthalmoplegia, no upward gaze but limited downward gaze. A muscle biopsy showed variability in muscle fiber size, scattered ragged red fibers and 10-20% fibers showing cytochrome c oxidase deficiency. Genetic analysis revealed biallelic *POLG* mutations: a novel c.590T>C; p.(F197S) mutation in the exonuclease domain and c.2740A>C; p.(T914P) mutation in the polymerase domain.

Deep sequencing identified multiple large-scale mtDNA rearrangements and a small increase in somatic point mutations. Biochemical *in vitro* analyses of the mutant proteins showed that p.(T914P) had no activity but the p.(F197S) resulted in an active protein with reduced exonuclease and polymerase activities.

Our study confirms the pathogenicity of the mutations and expands the molecular understanding of the patient's phenotype.

P15 - Structural basis of mitochondrial transcription

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The mitochondrial genome is transcribed by a dedicated single-subunit mitochondrial RNA polymerase (mtRNAP), which also generates the RNA primers required for DNA replication. Unlike the related T-odd bacteriophage RNA polymerases, mtRNAP depends on auxiliary protein factors for each step of the transcription cycle. How these factors act in concert with mtRNAP to facilitate mitochondrial gene expression, however, remains poorly understood. We have determined the structure of the human mitochondrial transcription initiation complex, which reveals how the two initiation factors TFAM and TFB2M facilitate promoter binding and DNA opening, respectively. Furthermore, we have solved structures of the mitochondrial transcription elongation factor TEFM in isolation and bound to the transcribing polymerase in an anti-termination complex, which demonstrate how TEFM interacts with both the nucleic acid and the polymerase to facilitate processive transcription. These results elucidate the mechanistic basis of transcription initiation and processive elongation in human mitochondria and provide the framework for studying the regulation of mitochondrial gene transcription. Together with previous data, a structural view of the transcription cycle in mitochondria emerges that illustrates how functionally unrelated protein folds were adopted for a role in this unique transcription system.

P16 - More than mitochondrial fusion: Role of Mitofusin 2 in cellular stress response

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Mitofusins are key players in the control of mitochondrial dynamics, having an active role on the regulation of fusion between two of these organelles. Mitochondrial dynamics is essential for the maintenance of healthy mitochondria, which in turn are of unique importance for overall cellular homeostasis. Mitofusin 2 (MFN2) plays a role in essential cellular processes such as mitophagy, apoptosis, lipid transfer and calcium homeostasis. Importantly, MFN2 point mutations cause Charcot-Marie-Tooth Type 2A (CMT2A), a peripheral neuropathy characterised by progressive loss of muscle tissue and touch sensation. MFN2 has also been linked with highly common neurodegenerative diseases, like Alzheimer's and Parkinson's. Finally, a role in common diseases of our aging population was also proposed, like cardiac defects, diabetes, liver deficiencies and cancer. The disease-underlying function of MFN2 was initially thought to be fusion-related. However, we and others have shown that there is no correlation between CMT2A disease mutants and mitochondrial fusion capacity. Based on our findings, we suggest that defects in stress response pathways, such as clearance of mitochondria by mitophagy or elimination of damaged cells by apoptosis may underline its role in CMT2A.

P17 - TRPV4 channels acts as a mitochondrial protein and is present in the ER-Mitochondrial contact sites

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Thermosensitive TRPV4 channel has been linked with the development of sensory defects, neuropathic pain, neurodegenerative disorders such as Charcot Marie Tooth disease and various muscular dystrophies. In all these cases mitochondrial abnormalities were tagged as cellular hallmarks and such abnormalities have been reported as a key factor for the pathophysiological conditions. Mitochondria also have the unique ability to sense and regulate their own temperature. Here, we demonstrate that TRPV4, localizes to a subpopulation of mitochondria in various cell lines and primary cells. Improper expression and/or function of TRPV4 induce several mitochondrial abnormalities such as low oxidative potential, high Ca2+-influx and changes in electron transport chain functions. TRPV4 is also involved in the regulation of mitochondrial morphology, smoothness, and fusion-fission events. The C-terminal cytoplasmic region of TRPV4 can localize it to mitochondria and interacts with mitochondrial proteins including Hsp60, Mfn1, and Mfn2. Further analysis confirms that TRPV4 is specifically present in the ER-mitochondrial contact sites which is relevant for several cellular functions. Regulation of ER-mitochondrial contact points by TRPV4 may contribute to previously uncharacterized mitochondria-specific functions observed in various cell types. This discovery may help to link TRPV4-mediated channelopathies with ER/mitochondria-mediated diseases.

P18 - Mitochondrial protein TMEM70: key role in the biogenesis of ATP synthase

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TMEM70 is a transmembrane protein localized in the inner mitochondrial membrane and involved in the biogenesis of ATP synthase, but its molecular role in this process is still unknown. *TMEM70* mutations cause isolated deficiency of ATP synthase often resulting in a fatal neonatal mitochondrial encephalocardiomyopathy. To understand molecular mechanism of TMEM70 action, we generated tamoxifen (TAM) inducible knockout.

The investigations showed more pronounced decrease of the fully assembled F_1F_o ATP synthase and accumulation of F_1 subcomplex in liver than in heart. This is likely due to the slower turnover of ATP synthase in heart tissue. Furthermore, we demonstrated the presence of alternated profile of ATP synthase subcomplexes in liver of TAM treated mice. Quantification of different OXPHOS proteins showed substantially decreased level of F_1-alpha and changed levels of other complexes in liver. We also observed defective utilization of mitochondrial membrane potential. Moreover, the oxygen consumption induced by addition of cytochrome c indicated damaged liver mitochondria of the treated mice. In addition, the oxidative stress and apoptosis were increased.

In conclusion, induction of *Tmem70* knockout impairs primarily liver function, and it resembles symptoms present during metabolic crises in patients. This contrasts with the primarily cardiologic presentation at disease onset in humans.

P19 - Three rare LHON pathogenic substitutions in patients with low mutation load

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Leber's hereditary optic neuropathy (LHON) is one of the most common maternally inherited mitochondrial diseases. It is characterized by a subacute bilateral loss of vision. LHON is caused by a mutation affecting mitochondrial complex I (CI). LHON mutations are typically homoplasmic but heteroplasmy, predominantly for the m.3460G>A, has been detected as well.

We present clinical, molecular and biochemical investigations of three patients with LHON caused by rare point substitutions in mtDNA. One patient had known mtDNA mutation (m.13513 G>A), the others had new variants (male with m.13379 A>G in *MT-ND5* gene and female with m.14597 A>G in *MT-ND6* gene). NGS analysis and Sanger sequencing revealed low mutation load in blood (24%, 47%, 25% respectively). Our data, including segregation analysis, measurement of ROS production and cytotoxic effect of paraquat and high-resolution respirometry, showed that nucleotide variant m.14597 A>G can be classified as a pathogenic mutation. More functional studies need to be done to understand the role of m.13379 A>G, because of the boundary results obtained from our experiments. No abnormalities were observed in the patient's fibroblasts with m.13513 G>A mutation what can be explained by low heteroplasmy load and as a result unexpressed biochemical defect of CI.

P20 - Impact of Apoptosis Inducing Factor deficiency on Harlequin mouse heart

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Introduction: Apoptosis Inducing Factor (AIF) is a mitochondrial flavoprotein with NADH oxidase activity, involved in the caspase-independent induction of apoptosis. The Harlequin mouse (Hq) presents a proviral insertion in the *AIFM1* gene, leading to variable degree of complex I deficiency in different tissues, and clinical symptoms including a higher risk of hypertrophic cardiomyopathy.

Aim: study the altered cellular routes involved in the pathophysiology of the heart in Hq mice.

Methods: Proteins showing differential levels by iTRAQ analysis in heart homogenates of wild type (WT) and Hq hemizygous male (6 months-old, both groups) and authophagy markers were analysed by western blot.

Results: in comparison to WT, Hq mice showed: i) lower levels of the sarcomeric proteins Regulatory Myosin Light Chain 3 (MYL3), cardiac Troponin C and Troponin I; ii) higher levels of Peroxirredoxin 6 and Annexin A3; and iii) higher levels of the autophagy markers LC3II, P62, Beclin1, ATG16L, and phosphorylated ULK1 and mTOR.

Conclusions: The present data suggest that AIF deficiency leads to disturbances in the sarcomere structure as well as an altered autophagy in the heart, which could be due to higher levels of oxidative stress and/or Annexin A3.

P21 - DNMT1 mutations leading to neurodegeneration paradoxically reflect on mitochondrial metabolism

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ADCA-DN and HSN-IE are rare neurodegenerative syndromes caused by dominant mutations in the replication foci targeting sequence (RFTS) of the DNA methyltransferase 1 (*DNMT1*) gene. Both phenotypes resemble mitochondrial disorders and mitochondrial dysfunction was first observed in ADCA-DN.

To explore mitochondrial involvement we studied the effects of *DNMT1* mutations in fibroblasts from four ADCA-DN and two HSN-IE patients. We document impaired activity of purified DNMT1 mutant proteins, which in fibroblasts results in increased DNMT1 amount, in turn leading to detection of the protein within mitochondria. However, mitochondrial DNA failed to show meaningful CpG methylation. Strikingly, we found activated mitobiogenesis and OXPHOS with significant increase of H_2O_2, sharply contrasting with a reduced ATP content. Metabolomics profiling of mutant cells highlighted purine, arginine/urea cycle and glutamate metabolisms as the most consistently altered pathways, similar to primary mitochondrial diseases. The most severe mutations showed activation of energy shortage AMPK-dependent sensing, leading to mTORC1 inhibition.

We propose that *DNMT1* RFTS mutations deregulate metabolism lowering ATP levels, as the result of increased purine catabolism and urea cycle pathways. This is associated with a paradoxical mitochondrial hyper-function and increased oxidative stress, possibly resulting in neurodegeneration in non-dividing cells.

P22 - De novo serine synthesis is protective in mitochondrial myopathy

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Dominant mutations in mitochondrial DNA (mtDNA) replicase Twinkle cause adult-onset mitochondrial myopathy with accumulation of mtDNA deletions in patients and in mice. The disease induces integrated mitochondrial stress response (ISRmt) in the affected tissues in humans and mice, with upregulated metabolic cytokines, mitochondrial folate cycle and imbalanced one-carbon metabolism. Furthermore, glycolytic flux diverts to de novo serine biosynthesis, driving glutathione synthesis. Whether serine biosynthesis affects disease progression is unknown.

To examine the role of serine biosynthesis in mitochondrial myopathy, we pharmacologically (NCT-503 compound) inhibited phosphoglycerate dehydrogenase (PHGDH), the rate-limiting enzyme for the pathway. In the muscle, PHGDH inhibition promoted muscle pathology and ISRmt, mTOR activity and eIF2α phosphorylation, but did not affect mtDNA deletion amounts. Serine-driven phospholipid synthesis was impaired. In vitro experiments indicated that PHGDH inhibition decreased oxidative capacity, and if chronic, compromised cell viability.

In conclusion, in the postmitotic skeletal muscle with mitochondrial dysfunction, the upregulated de novo serine synthesis, modifying cellular redox environment via increased glutathione synthesis, is protective. This finding suggests boosting of PHGDH activity as a therapeutic approach.

P23 - A CRISPR-Cas9 genetic screen reveals resistance mechanisms to the inhibitor of mitochondrial transcription in cancer cells

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Alterations in mitochondrial function are one of the hallmarks of cancer cells and targeting mitochondria has become a promising therapeutic approach for tumour treatment. We recently participated in the development of a novel class of anti-cancer drugs that specifically targets mitochondrial gene expression. These inhibitors of mitochondrial transcription (IMTs) exploit a strong antitumor activity without signs of acute or long-term toxicity *in vivo* (Bonekamp *et al.,* submitted). However, the development of chemoresistance is a very common phenomenon and constitutes a risk that could influence IMTs' therapeutic applications. In this study, we investigated the cellular mechanisms responsible for acquired resistance to IMT treatment in cancer cells. To this end, an IMT-resistant RKO cell line was generated and the factors influencing the sensitivity to the inhibitor were elucidated through a genome-wide CRISPR-Cas9 screen. The identification of mechanisms of increased sensitivity or acquired resistance to the inhibition of mitochondrial transcription revealed new possible targets for pharmacological interventions. Moreover, the identified mechanisms could potentially be used to understand the often-puzzling manifestations of mitochondrial diseases.

P24 - The in vivo role of the mitochondrial DNA nuclease MGME1

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Replication of mammalian mitochondrial DNA (mtDNA) is an essential process that requires high fidelity and control at multiple levels to ensure proper mitochondrial function. Mutations in the mitochondrial genome maintenance exonuclease 1 (*MGME1*) gene were recently reported in patients with mitochondrial disease. To study MGME1 function, we generated *Mgme1* knockout mice and reported that in addition to its roles in flap removal and processing of the 5' end of 7S DNA this protein might have regulatory role at the end of the mtDNA control region.

MGME1 homozygous knockouts mice develop mtDNA depletion and accumulate long linear subgenomic mtDNA molecules in range of different mouse tissues. Even though MGME1 nuclease activity was recently implicated in turnover of linear mtDNA molecules, MGME1 knockout tissues do not accumulate linear subgenomic fragments with age suggesting that other nuclease is crucial to degrade those species.

P25 - The in vivo role of RNaseH1

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Replication and maintenance of mitochondrial DNA (mtDNA) are essential cellular processes whose dysfunction leads to replication stalling, accumulation of mtDNA mutations and mtDNA copy number depletion, ultimately causing mitochondrial diseases. Therefore, understanding the precise mechanism of mtDNA replication and its underlying regulatory mechanisms is of fundamental importance. Recently, mutations in ribonuclease H1 *(RNASEH1)* gene were reported in patients with mitochondrial diseases. It has been suggested that RNaseH1 is involved in replication primer processing and removal as well as in directing origin-specific initiation of mtDNA replication.

To study *in vivo* role of RNaseH1 we generated and analysed *Rnaseh1* conditional knockout mouse. Our results demonstrate that RNaseH1 is essential for mouse embryonic development. Tissue specific heart and skeletal muscle knockout of *Rnaseh1* results in profound mtDNA depletion followed by decreased steady-state levels of mitochondrial transcripts and altered levels of OXPHOS complexes. Our data show that loss of function of RNaseH1 *in vivo* leads to severe mitochondrial dysfunction and support RNaseH1 involvement in mtDNA replication.

P26 - Reversible anaplerosis induced by mitochondrial dysfunction reveals metabolic plasticity in degenerating neurons

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Progressive neurodegeneration is a major manifestation of many mitochondrial disorders, underscoring the essential role of mitochondria in neuronal energy metabolism. Neurodegeneration is considered to be irreversible because of limited metabolic plasticity in neurons, yet little is known about the cell-autonomous implications of mitochondrial dysfunction for neuronal metabolism *in vivo*. Here, we report that mitochondrial dysfunction in Purkinje neurons lacking mitochondrial fusion triggers a profound rewiring of their proteomic landscape culminating in the sequential activation of precise metabolic programs that precede cell death. In particular, we identified the induction of TCA cycle anaplerosis to be a specific and conserved metabolic hallmark of early neuronal OXPHOS dysfunction. Surprisingly, restoration of mitochondrial fusion in OXPHOS-deficient Purkinje neurons with profound metabolic changes was sufficient to revert these responses and prevent cell death. These findings pave the way for developing targeted interventions to restore OXPHOS and preserve neuronal viability even at advanced stages of disease.

P27 - The structural basis of cristae shaping in Toxoplasma mitochondria

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Mitochondrial ATP synthase plays a crucial role in the maintenance of membrane morphology and membrane potential in mitochondria. It is generally accepted that by forming dimers, it shapes mitochondrial cristae, which is essential for organelle function. The transmembrane subunit *a* was shown to be responsible for proton translocation and underlying the dimeric structure. The human parasite *Toxoplasma gondii* has unique club-shaped cristae morphology and a minimal subunit *a.* To understand how the ATP synthase shapes cristae in *T. gondii*, we characterized it through a combination of cryo-EM, subtomogram averaging and electron cryo-tomography of whole mitochondria. We show that the native arrangement of the *T. gondii* ATP synthase is a hexamer, which is unprecedented in any known ATP synthase. The hexamer is composed of three dimers with no involvement of subunit *a* in dimer formation. On the supramolecular level, multiple hexamers are arranged around the cristae apices, thereby imposing membrane curvature. The unique *T. gondii* ATP synthase structure is dictated by 17 phylum-specific subunits and numerous bound cardiolipins, which we report for the first time. Thus, our results show that *T. gondii* has evolved a highly divergent macromolecular ATP synthase arrangement that provides the structural basis for its unique cristae morphology.

P28 - Investigating nucleoside supplementation in mtDNA depletion syndromes

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Mitochondrial DNA (mtDNA) depletion syndromes (MDDS) are a group of clinically heterogenous diseases characterized by a tissue specific depletion of mtDNA. Mutations in nuclear genes encoding proteins involved in mtDNA nucleotide pool maintenance, such as *DGUOK*, *TK2* and *RRM2B*, or mtDNA replication machinery including *POLG* and *TWNK* are the main contributing factors in MDDS. Patients don't survive beyond the first few years of life and little advancement has been made in terms of treatment for affected individuals. Building on previous cell culture studies we have been investigating if nucleoside supplementation is a valid therapeutic approach to tackle MDDS resulting from *DGUOK* mutations. Using Crispr/CAS9 mutagenesis we created a zebrafish mutant with a frameshift mutation in *DGUOK* which encodes deoxyguanosine kinase (dGK), a vital rate limiting enzyme involved in mtDNA purine nucleotide pool maintenance. The homozygous animals exhibit significantly reduced mtDNA levels in brain and liver, mirroring the patient's presentation. When supplemented with purine nucleosides over a 3-week period we see a significant increase in liver mtDNA copy number rescuing to wild type levels. This suggests that nucleoside supplementation may be a possible therapeutic approach to treat MDDS and it would be worthwhile exploring it further in other models.

P29 - Dinucleotide degradation by REXO2 is required to maintain promoter specificity in mammalian mitochondria

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Oligoribonucleases are conserved enzymes that degrade short RNA molecules of up to 5 nt in length, and are assumed to constitute the final stage of RNA turnover. We have investigated the *in vivo* function of the human oligoribonuclease REXO2, which localises to the mitochondria and cytosol. We find that REXO2 is specialised for the degradation of dinucleotides, and shows no preference between RNA and DNA dinucleotide substrates. This specificity is supported by the crystal structure of substrate-bound REXO2, which forms an obligate homodimer. A heart and skeletal muscle-specific knockout mouse displays elevated mitochondrial dinucleotide levels and alterations in mitochondrial gene expression patterns indicating that elevated levels of dinucleotides prime transcription initiation *in vivo*. We find that dinucleotides act as potent stimulators of mitochondrial transcription initiation using a reconstituted *in vitro* system, from both mitochondrial promoters and from non-canonical sites. Our data demonstrate that increased levels of dinucleotides short-circuit the formation of the first phosphodiester bond during transcription initiation. Efficient RNA turnover by REXO2 is thus required to maintain promoter specificity and ensure correct regulation of transcription in mammalian mitochondria.

P30 - A novel mitochondrial assembly factor RTN4IP1 has an essential role in the final stages of Complex I assembly.

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Optic neuropathies are neurodegenerative disorders frequently caused by variants in nuclear-encoded mitochondrial genes. Whole-exome sequencing identified *RTN4IP1*, encoding a Reticulon-4-Interacting Protein 1, as a disease gene causing isolated optic atrophy, lactic acidosis, hypotonia and early-onset encephalopathy[1]. Patient cells harbouring compound heterozygous pathogenic variants in *RTN4IP1* showed a complete loss of RTN4IP1 protein and a severe Complex I (CI) assembly defect. We generated a CRISPR/Cas9-mediated *RTN4IP1* knockout human cell line to further investigate the molecular basis of RTN4IP1. Western blotting, Blue-native PAGE, complexome profiling analysis and high-resolution respirometry was used to assess the role of RTN4IP1 in mitochondrial function. In addition, RTN4IP1-interacting proteins have been identified by immunoprecipitation and mass spectrometry analysis. Characterisation of CRISPR/Cas9 *RTN4IP1* knockout cells showed a marked decrease in the steady-state levels of the mature CI and its subunits. Complexome profiling of the *RTN4IP1*-knockout cells revealed an abnormal assembly of the CI N-module and P_{ND5}-module as well as a complete absence of the FOXRED1 assembly factor in CI intermediates. In addition, we also detected a marked reduction in the respiratory chain supercomplexes. Our data suggest that *RTN4IP1* encodes a novel assembly factor that has an essential role in the late stage of mitochondrial CI assembly pathway.

[1] Charif M *et al.* Neurologic Phenotypes Associated With Mutations in *RTN4IP1* (O*PA10*) in Children and Young Adults. *JAMA Neurol.* 2017

P31 - Mitochondrial DNA rearrangements in inclusion body myositis characterized by deep sequencing

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Inclusion body myositis (IBM) is the most common acquired myopathy in individuals over 50 years and is characterized by endomysial inflammatory cell infiltration, rimmed vacuoles, and frequent cytochrome c oxidase deficient muscle fibers. Previous studies have demonstrated clonal expansion of large-scale mitochondrial DNA (mtDNA) deletions in muscle fiber segments explaining the cytochrome c oxidase deficiency.

By deep sequencing of skeletal muscle mtDNA, with a mean depth of coverage of 45,000x, we performed detailed mapping of mtDNA in 21 patients with IBM and 11 age-matched controls.

Multiple mtDNA deletions and also duplications were identified in all IBM patients and controls at a stringent clustering threshold of minimum 5 reads supporting a breakpoint. The IBM patients demonstrated in general a high level of large-scale rearrangements with a mean level of heteroplasmy of 10% (range 1-35%) compared to controls (1%, range 0.18-3%). There were also a small number of heteroplasmic point variants in coding regions, with low levels of heteroplasmy, indicating that they were somatic mutations. The frequency of point mutations did not differ significantly between the groups.

This detailed mapping of somatic mtDNA rearrangements in IBM may serve as a basis for further studies to unravel their pathogenesis.

P32 - Force spectroscopy study of MTERF1 as a polar roadblock

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Recent *in vitro* experiments suggest that MTERF1 acts as a polar roadblock, with a permissive and non-permissive orientation towards both transcription and replication.

We employed a single-molecule approach using magnetic tweezers and DNA hairpins to trigger DNA unwinding in absence of molecular motors. We characterized MTERF1 lock formation from the permissive and non-permissive orientation. We showed that: (1) lock duration depends on the termination site orientation and (2) that DNA unwinding alone (in absence of molecular motors) is sufficient to observe MTERF1 triggered lock. Our study helps to better understand the mechanism behind MTERF1 polar blocking of motor enzymes. But also describes the methodology of single-molecule studies that can be extrapolated to the study of other actors of mitochondrial DNA processing.

P33 - Copy-choice recombination during mitochondrial L-strand synthesis causes DNA deletions.

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Mitochondrial DNA (mtDNA) deletions are associated with mitochondrial disease, and also accumulate during normal human ageing. The mechanisms underlying mtDNA deletions remain unknown although several models have been proposed. Here we use deep sequencing to characterize abundant mtDNA deletions in patients with mutations in mitochondrial DNA replication factors, and show that these have distinct directionality and repeat characteristics. Furthermore, we recreate the deletion formation process in vitro using only purified mitochondrial proteins and defined DNA templates. Based on our in vivo and in vitro findings, we conclude that mtDNA deletion formation involves copy-choice recombination during replication of the mtDNA light strand.

P34 - Mutations in the mitochondrial RNA polymerase POLRMT are associated with a spectrum of clinical presentations

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Many mitochondrial disorders result from mutations in components of the nuclear-encoded mitochondrial DNA (mtDNA) replication machinery and to date more than 200 disease causing mutations have been identified in the catalytic subunit of mitochondrial DNA polymerase γ. In contrast, the role of the mtDNA transcription machinery in mitochondrial disease remains relatively unknown and no mitochondrial diseases have been associated with POLRMT, the RNA polymerase responsible for transcription of the mitochondrial genome. Using whole-exome sequencing, we have identified novel recessive and dominant POLRMT variants associated with mitochondrial disease in nine individuals. Functional characterisation of patient fibroblasts revealed a defect in mitochondrial mRNA synthesis, although no mtDNA deletions or copy number abnormalities were identified. Mild decreases in the levels of both OXPHOS subunits and fully-assembled complexes were observed *in vivo*, whilst functional *in vitro* characterisation of recombinant POLRMT variants revealed that patient mutations exhibited variable, but deleterious effects on mitochondrial transcription. Our results demonstrate, for the first time, that pathogenic variants in the *POLRMT* gene can cause a spectrum of clinical phenotypes ranging from childhood-onset developmental delay, speech/intellectual disabilities, hypotonia and growth defects to late-onset PEO and muscle weakness and emphasise the importance of defective mitochondrial transcription as a disease mechanism.

P35 - Niacin supplementation alleviates disease symptoms in patients with mitochondrial myopathy

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The most frequent form of adult-onset mitochondrial disorders is mitochondrial myopathy, a progressive muscle disease with no curative treatment. Supplementation with an NAD^+-precursor vitamin B3-form, nicotinamide riboside, delayed mitochondrial myopathy in mice. Here, we tested the effect of NAD^+-boosting in mitochondrial myopathy patients.

We recruited five PEO patients with single or multiple mtDNA deletions, and 10 controls - two gender- and age-matched controls for each patient – and supplemented them with niacin, an NAD^+-precursor and a vitamin B3-form. We analyzed the effects of niacin by clinical, blood, functional and molecular analyses (metabolomics, RNAseq) of muscle and blood at the initiation of the study, at 4 and 10-month time points.

When entering the study, the patients showed NAD^+ depletion both in the whole blood and muscle samples, rescued by niacin. Niacin also improved muscle respiratory chain function and mitochondrial mass, and increased exercise capacity and core muscle strength in the patients. Metabolomics and transcriptomics analyses showed major niacin-related changes. We conclude that mitochondrial myopathy is associated with NAD^+ depletion in both blood and muscle. Furthermore, niacin acts as an efficient NAD^+ booster and metabolic modifier, restoring NAD^+ levels and improving muscle mitochondrial biogenesis and function in mitochondrial myopathy irrespective of patients' age.

P36 - Nuclear regulation of mitochondrial tRNA production

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The size and organization of the has been compacted significantly since its endosymbiosis from an α-proteobacterial ancestor. This compaction has necessitated the evolution of unique mechanisms to facilitate rapid changes in gene expression in response to the changing energy demands of the cell. Since the mitochondrial genome encodes only a subset of the proteins that make up mitochondria, the coordinated regulation of the mitochondrial transcriptome by the nucleus is of particular importance for the maintenance of cell health and energy metabolism. We have investigated the unusual features of mitochondrial tRNAs and the RNA-binding proteins that control their production, maturation, translation and stabilization to understand the regulation of mitochondrial gene expression and its contribution to health and disease. I will highlight the devastating consequences of dysregulated mitochondrial gene expression and protein synthesis in new models of disease caused by genetic disruption of tRNA-binding proteins. These mouse models of disease have enabled us to understand the in vivo role of fundamental processes that regulate mitochondrial tRNA metabolism and the pathogenesis of diseases caused by impaired protein synthesis. This work illustrates that balanced mitochondrial tRNA production is required for the regulation of nuclear gene expression, as well as translation in multiple cellular compartments.

P37 - Making sense of nonsense mutations in paediatric primary mitochondrial diseases

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Introduction. Nonsense mutations account for ~12% of Mendelian disorders, including many mitochondrial disorders, for which there are no curative therapies. A potential therapeutic strategy is to pharmacologically target nonsense mutations by inducing translational read-through or by inhibiting nonsense-mediated decay. Clinical trials for translational read-through therapy have had variable success in other disorders. The present study aimed to test the efficacy of translational read-through inducing drugs (TRIDs) and nonsense-mediated decay inhibitors (NMDis) in fibroblasts from paediatric patients with mitochondrial diseases.

Methods. 19 fibroblast lines, carrying bi-allelic nonsense mutations, were treated with either TRIDs (Gentamicin, PTC124, or RTC13) and/or an NMDi (Amlexanox). Transcript and protein analysis acted as initial cellular readouts. Functional studies in progress aim to determine whether treatment would improve disease phenotype.

Results. Of the 19 patient cell lines, seven have displayed positive responses to treatment detected by qPCR and immunoblotting. Ongoing functional studies aim to determine if these increased expression levels translate into gain-of-function.

Discussion. Our study has demonstrated proof-of-principle evidence for the use of translational read-through therapy to treat mitochondrial disorders. Functional studies will determine if increased expression levels correlate with an increase in function sufficient to ameliorate mitochondrial disease phenotypes *in vitro*.

P38 - Mitochondrial Involvement In A Patient With Neurologigal Disease With Two Novel Mutations In A Compound Heterozygous In EXOSC8 Gene

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We report the clinical and biochemical findings from a patient who suffer a slowly progressive neurodegenerative disease and complex I and III deficiency of the mitochondrial respiratory chain in muscle. The mtDNA analysis by Southern blot (deletions, depletion) was normal, and the whole mtDNA sequencing did not show any pathological variant. Whole exome sequencing (WES) uncovered three mutations (two of them novel) in a compound heterozygous in EXOSC8 gene (NM_181503.3: c.[390delG];[628C>T;815G>C];p.[(Lys130Asnfs28ter)];[(Pro210Ser;Ser272 Thr)]) that encodes exosome complex component RRP43 protein (EXOSC8). EXOSC8 is an essential protein of the exosome core. The exosome is a multi-protein complex required for the degradation of AU-rich element (ARE) containing messenger RNAs. Currently, only two homozygous mutations in EXOSC8 have been described as the cause of a progressive and lethal neurological disease. Furthermore the reduced expression of EXOSC8 may have an effect on mitochondrial function, possibly due to disturbed mRNA processing of mitochondrial genes containing ARE elements as has been suggested. To confirm the importance of described mutations in the neurological symptoms that the patient reports, we have realized “in silico” and experimental expression studies in RNA and protein of EXOSC8. Accordingly, the relationship between EXOSC8 and mitochondrial genes containing ARE elements should be investigated.

P39 - Metabolic adaptation upon disruption of mitochondrial SAM import

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Methylation is an integral part of numerous cellular processes, such as epigenetic regulation, biosynthesis and maturation of metabolites and proteins. Several methyltransferases are localized to mitochondria, which catalyse the transfer of methyl groups from S-Adenosylmethionine (SAM) to their target. However, the global requirements for methylation within mitochondria are poorly mapped and understood. In a comprehensive genetic, molecular and computational approach, we gradually disrupted the import of SAM through SLC25A26 in fruit flies, which is the only known transporter for SAM across the inner mitochondrial membrane. We have defined clusters of proteins that react to mitochondrial SAM deficiency in an ambivalent mode. Whereas metabolic changes aiming at increasing the cytosolic SAM pool are part of an early response, mitochondrial metabolite levels only become affected at a profoundly decreased methylation potential. By mapping the intra-mitochondrial protein methylome, we are identifying the central SAM-requiring components in the identified protein clusters. With regard to alterations in one-carbon metabolism and SAM levels in a series of diseases, our study defines key proteins in mitochondria that require SAM-dependent methylation for their function.

P40 - Atomic model of human mitoribosome

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Human mitoribosomes carry out the synthesis of proteins that form part of the oxidative phosphorylation pathway. They vary from their cytosolic counterparts not only in protein and RNA content, but also have specific modifications that are important for stability and correct assembly. We determined the structure of the human mitoribosome at 2.6 Å resolution using single-particle cryo-EM, which allowed visualization of RNA modifications, including base and sugar methylations and pseudouridylations. We confirmed the presence of the previously identified mt-rRNA modifications in 12S rRNA of the small subunit (mt-SSU), 16S rRNA of the large subunit (mt-LSU), and mt-tRNAVal. Additional densities might correspond to unreported sites of mt-rRNA modifications. Furthermore, from the density map we identified several previously unreported ligands, including nucleotides, polyamines and 2Fe-2S clusters. Three 2Fe-2S clusters observed, were shared between mS25-bS16m, bS6m-bS18m, uL10m-bs18a. Finally, the improved resolution revealed that mS29 at the subunit interface binds ATP rather than GDP as previously suggested, and a new GDP-binding site is identified at a distinct location. Taken together our work represents the most comprehensive description of the structure of human mitoribosome.

P41 - TK2 deficiency. The importance of laboratory diagnostics in the era of targeted therapy

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Inherited neuromuscular diseases manifesting in the first year of life present with severe symptoms with failure to thrive, profound muscle atrophy and early death. Incidence of some forms of these disorders, including the most frequent SMA type I could exceed 1:10000. On time molecular diagnosis is very important as novel targeted therapy appear rapidly last years. Deficiency of thymidine kinase 2 leads to the presentation of several phenotypes, one of which resembles spinal muscle atrophy. *TK2* gene encodes the enzyme thymidine kinase, which is involved in the biogenesis of mitochondrial nucleotide pools. Lack of nucleotides leads to replication of only a limited number of copies of mtDNA in mitochondria. Today nucleosides bypass therapy is promising method of treatment. Initially we found 2 unrelated patients with *TK2* mutations on NGS targeted genes panel, following Sanger sequencing of *TK2* gene in another patient with high suspicious phenotype. Having analyzed literature data we performed Sanger sequencing of all *TK2* gene exons in 96 patients under 1 year old with SMA phenotype but lacking the frequent deletion in *SMN1* gene. Two additional patients with *TK2* mutations were revealed by the screening. Altogether in 5 patients we revealed 6 mutations, 4 of which are novel.

P42 - Pathways and players in respirasome biosynthesis

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Over the past sixty years, researchers have made outmost efforts to clarify the structural organization and functional regulation of the four complexes I to IV (CI to CIV) that configure the mitochondrial respiratory chain. As a result, the composition of each individual complex is practically known and, aided by notable structural advances in mammalian mitochondria, it is now widely accepted that these complexes stablish interactions to form higher-order supramolecular structures called supercomplexes (SCs) and respirasomes (or SC I+III_2+IV_n). However, the mechanistic roles and players that regulate the function and biogenesis of such superstructures are still under intense debate. Noteworthy, understanding the pathways involved in the assembly and organization of respiratory chain complexes and SCs is of high biomedical relevance because molecular alterations in these pathways frequently result in severe mitochondrial encephalomyopathies and cardiomyopathies.

Biochemical analyses in a diversity of OXPHOS-deficient cellular and animal models support the idea that CI function relies on the assembly and stability of the respirasomes, and the COX7A protein family has been proposed to be essential for this process. To gain insight into the mechanisms and players that regulate respirasomes biogenesis, we have developed a combined experimental strategy based on the genetic manipulation of *COX7A* genes expression in human cultured cell lines. Our data provide experimental evidence for the co-existence of diverse SC species whose biosynthesis is alternatively determined by different COX7A isoforms, which are essential regulators of respiratory chain function.

P43 - Cardiac specific deletion of MTP18 induces heart failure in mice

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The functions of mitochondria are intimately linked to their form, which is regulated by a balance of membrane fusion and fission. Dynamin-like GTPases OPA1 and MFN1/2 perform fusion of the inner and outer membranes, respectively while cytosolic DRP1 regulates membrane fission by binding to the outer membrane of mitochondria at dedicated receptors to drive scission. In this study, we investigated the putative mitochondrial fission factor MTP18, which has been previously linked to cardiac homeostasis. We demonstrate that MTP18 is an inner membrane protein whose ectopic induction drives DRP1-dependent mitochondrial fragmentation. In cultured cells, depletion of MTP18 causes an elongation of the mitochondrial network and renders cells more sensitivity to programmed cell death. Longitudinal echocardiographic analyses of mice deleted of MTP18 in cardiomyocytes of mice (Myh6-Cre; $MTP18^{Flox/Flox}$) reveals a progressive dilated cardiomyopathy and heart failure by middle age. At a young age, KO mice display no cardiac abnormalities however transcriptomic, metabolomic, and bioenergetic studies point to cardiac metabolic rewiring and the induction of a sterile inflammatory response at the onset of cardiomyopathy. We propose that MTP18 may act as an inner membrane scaffold cellular homeostasis and that it is important in the protection against heart failure.

P44 - dNTP levels determine the frequency and identity of ribonucleotides in mitochondrial DNA

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Incorporation of ribonucleotides into DNA during genome replication is a significant source of genomic instability, and defects in their removal are associated with human disease. The frequency of ribonucleotides in DNA is determined by dNTP/rNTP ratios, by the ability of DNA polymerases to discriminate against ribonucleotides, and by the capacity of repair mechanisms to remove incorporated ribonucleotides. Interestingly, the nuclear DNA and the mitochondrial DNA (mtDNA) differ with regard to ribonucleotide content. To simultaneously compare how the nuclear and mitochondrial genomes incorporate and remove ribonucleotides, we challenged these processes by altering the pool of cellular dNTPs.

We discovered an inverse relationship between the concentration of dNTPs and the amount of ribonucleotides incorporated in mitochondrial DNA, while in nuclear DNA the ribonucleotide pattern was only altered in the absence of ribonucleotide excision repair. Our analysis uncovers major differences in ribonucleotide repair between the two genomes and provides concrete evidence that both budding yeast and mouse mitochondria lack mechanisms for efficient removal of single ribonucleotides incorporated by the mtDNA polymerase.

P45 - The function of mitochondrial transcription factor A in the compaction of mitochondrial DNA into nucleoids

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Mitochondrial transcription factor A (TFAM) has an essential role in regulation of mitochondrial DNA (mtDNA). It's role as a transcription factor is to recruit the RNA polymerase (POLRMT) and transcription factor B2 (TFB2M) to initiate transcription and expression of the mitochondrial genome. TFAM is found to be the most abundant component of the nucleoid. We have previously demonstrated that TFAM controls mitochondrial transcription and replication by compaction of mtDNA into nucleoids. At physiological ratios of TFAM, large variations of compaction can be seen, from fully compacted nucleoid structures to naked DNA, thus displaying epigenetic-like control. The mechanisms of how TFAM regulates the number of active copies of mtDNA are still unclear.

There is evidence that the TFAM C-terminal tail is of major importance in the ability of TFAM to compact the mtDNA sufficiently to inhibit DNA replication and gene transcription. In this study we have purified numerous recombinant TFAM mutants to investigate TFAM as a regulator of the mitochondrial genome. Furthermore, a variant of TFAM with a homozygotic single-point mutation has been found to cause neo-natal liver failure due to mtDNA depletion, leading to fatality in infants. Here we examine this mutant's ability to bind and compact DNA *in vitro*.