S9.5 The mitofusin of *D. melanogaster*: Genetic and functional analysis to understand pathogenesis of Charcot-Marie-Tooth IIa

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Mitofusins (Mfns) are outer mitochondrial membrane GTPases which control mitochondrial fusion and morphology. Mammals possess two different Mfns (Mfn1 and 2) which do not seem to display redundant functions. While mitochondrial fusion mediated by the inner mitochondrial membrane GTPase OPA1 requires MFN1, MFN2 appears to play roles outside mitochondria in the regulation of metabolism and cell growth. Moreover, MFN2 has been involved in several diseases including the peripheral Charcot-Marie-Tooth type IIa (CMTIIa) neuropathy. Both MFN1 and MFN2 are essential for embryonic development and mice deficient in either gene die in mid-gestation. Thus, there are no animal models to analyze MFN2 function *in vivo*. We decided to use *Drosophila melanogaster* to dissect the role of Mfn *in vivo* and to find its genetic interactors. *Marf*, the Mfn homologue of *Drosophila*, displays 47% of identity with both Mfns. Expression of MARF in both *Mfn1* and *Mfn2*$^{-/-}$ mouse embryonic fibroblasts rescues the deficient mitochondrial fusion, indicating that it can substitute for both Mfns. Knock-down of *Marf* in the nervous system induces muscular plaque defects at the third instar larva stage, with lack of mitochondria in the distal axon. The overexpression of Mfn2 in fruitflies is larval lethal, causing clusterization of mitochondria in the perinuclear regions of neuronal cell bodies. On the other hand, fruitflies expressing Mfn1 can develop into adults even if they display locomotory defects. We will discuss the role of *Marf* in the control of mitochondrial dynamics, in order to understand its involvement in the function of the larval nervous system.

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S9.6 LHON mutations and rotenone induce the unfolded protein response in human neural cells

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The aim of this study was to understand the transcriptional and biochemical consequences of complex I inhibition by LHON (Leber's Hereditary Optic Neuropathy) mutations and rotenone. LHON is characterized by death of retinal ganglion cells and demyelination. A microarray study of mitochondrial disease focused on LHON demonstrated an activation of transcripts of the Unfolded Protein Response (UPR), including ATF4, ATF3, BiP and CHOP (Cortopassi et al., 2006), and an inhibition of myelin-related transcripts. LHON mutations affect complex I, and so we reconstructed these effects with rotenone. Exposure of myelinating cells to rotenone also induced UPR transcripts and inhibited myelin transcripts and myelinogenic differentiation. Knockdown of OPA1 and Mitofusin 2 induced ATF3 and its transcriptional targets in common with LHON mutations. At the protein level, the identical concentration of rotenone that inhibited ATP synthesis significantly induced the phosphorylation of the UPR proteins PERK, eIF2alpha, and ATF4 protein levels. The phosphorylation of PERK, an endoplasmic reticular stress kinase, as a result of mitochondrial inhibition further supports a connection between mitochondrial inhibition and ER stress signaling. Thus, ATF3 and its targets are induced in common by LHON mutations, OPA1 or MFN2 knockdown, and the UPR pathway is induced by LHON mutations and rotenone. We are currently testing the dependence of complex I-dependent stasis and death in cell models of LHON on the UPR pathway.

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S9.7 Dominant optic atrophy caused by a novel *OPA1* mutation: Disruption of the mitochondrial network with preserved bioenergetics

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The aim of this study was to determine the pathogenetic mechanism of autosomal dominant optic atrophy (ADOA). Bioenergetic failure, abnormalities of the mitochondrial network and increased susceptibility to apoptosis have all been proposed as possible pathogenetic mechanisms. However, the prominent susceptibility of the retinal ganglion cell in this disease remains unclear. We report the clinical features of an ADOA family with a novel deletion of *OPA1* gene in the GTPase domain, and investigate mitochondrial morphology and bioenergetics in cells derived from these patients. Muscle biopsy showed neurogenic atrophy and abnormal distribution of mitochondria. Confocal microscopy revealed increased mitochondrial fragmentation in fibroblasts. In differentiated myotubes, mitochondria were unevenly distributed, with clustered organelles alternating with areas of mitochondrial dearth. These abnormalities were not associated with altered bioenergetics or increased susceptibility to pro-apoptotic stimuli. The observed mitochondrial network disruption appeared a primary event. This phenomenon, independently of bioenergetics defects, might provide an explanation for the predominant retinal ganglion cell degeneration, whose function may depend on the fine positioning of mitochondria in the axons.

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S9.8 Mitofusin foci: Endogenous localization and apoptotic behavior

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Mitochondria are dynamic organelles that undergo frequent fission and fusion events. The mitofusions, Mfn1 and Mfn2, mediate mitochondrial fusion. These proteins localize to distinct areas on the mitochondria and have been shown to tether the mitochondria together in both hetero- and homo-dimeric complexes. The aim of this study was to further characterize the mechanism by which the mitofusins promote fusion, focusing on endogenous localization,

importance of the GTPase domain and behavior during apoptosis. Using immunofluorescence, we found that endogenous Mfn2 is enhanced in specific regions on the mitochondria, particularly at tips of mitochondria. Overexpressed mitofusin also forms distinct foci and we have found using FRAP (Fluorescence Recovery After Photobleaching) that mutants of mitofusins devoid of GTP binding display markedly different mobilities on the mitochondrial membrane reflecting changes in complex formation. During apoptosis the mitochondria fragment and fusion is inhibited. Interestingly, during apoptosis a mutant of Mfn2 that typically is incapable of forming foci moves into foci that coalesce with Bax, the pro-apoptotic Bcl-2 family member. Curiously, we have found that upon Bax translocation the mitofusins, along with Fis1 and OMP-25, lose mobility, indicating that the mitochondria outer membrane is undergoing additional changes not previously described.

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S9.9 MiD51 and MiD49: New mediators of mammalian mitochondrial distribution

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Mitochondria are organized into networks that are important for proper cellular function. These networks are regulated by fission and fusion events, as well as transport along cytoskeletal elements. The aim of this study was to characterize two novel mitochondrial proteins, *Mi*tochondrial *D*istribution protein 51 kDa (MiD51) and 49 kDa (MiD49), that appear to be involved in the regulation of mitochondrial morphology and distribution. Ectopic expression of both MiD51 and MiD49 in COS-7 cells results in two aberrant mitochondrial phenotypes — long extended tubules and peri-nuclear collapsed mitochondria. Live cell confocal microscopy was used to highlight the dynamic nature and connectivity of the mutant mitochondria by co-expression with the photoswitchable fluorescent protein mitochondrial-Dendra2. While mitochondrial movement in mammalian cells is principally driven by a connection with the microtubule network, these MiD proteins instead appear to induce an interaction between mitochondria and actin filaments. Only simultaneous knockdown of both endogenous proteins via RNAi, results in atypical mitochondrial distribution in both COS-7 and HeLa cells, as well as reduced HeLa cell viability. We therefore propose that MiD51 and MiD49 share functional redundancy in the distribution of mitochondria in mammalian cells, perhaps in regulation of actin/mitochondrial interactions.

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S9.10 Cell type specificity of mitochondrial dynamics: Striking differences between adult rat cardiomyocytes, HL-1 cells and human pancreatic cells

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The aim of this study was to analyze mitochondrial dynamics in adult rat cardiomyocytes, HL-1 cells and human pancreatic cells. Mitochondrial imaging was performed by real time confocal microscopy using mitochondria-specific fluorescent probes. The results revealed remarkable differences in mitochondrial dynamics, as well as in spatial arrangement of mitochondria in these cells, probably due to cell specific cytoskeleton organization. In adult rat cardiomyocytes, in which mitochondria are arranged regularly (crystal-like), no displacement of mitochondria was observed with only very small amplitude rapid vibration. In contrast, in primary human pancreatic and HL-1 cells we documented complex dynamic behaviour of mitochondria. The common types of mitochondrial dynamics observed were: 1) fission, fusion and small oscillatory movements of mitochondria; 2) larger movements including filament extension, retraction, and 3) fast oscillating branching in the mitochondrial network and fast long-distance intracellular translocation of single mitochondria or mitochondrial filaments. In summary, we show that mitochondrial dynamics may be very different in different cell types. These variations could be related to a significant role of cell specific integrations of mitochondria with other intracellular systems like cytoskeleton and ER.

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S9.11 Novel mechanism of elimination of malfunctioning mitochondria (mitoptosis): Formation of mitoptotic bodies and extrusion of mitochondrial material from the cell

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Energy catastrophe when mitochondria hydrolyze glycolytic ATP instead of producing it has been modeled. In highly glycolyzing HeLa cells 30–50% of the population survived after inhibition of respiration and uncoupling of oxidative phosphorylation for 2–4 days. The survival was accompanied by selective elimination of mitochondria. This program of mitoptosis includes (i) fission of mitochondrial filaments, (ii) clusterization of mitochondria in perinuclear area, (iii) occlusion of mitochondrial clusters by a membrane (formation of a "mitoptotic body"), (iv) decomposition of mitochondria to small vesicles, (v) protrusion of the body from the cell and (vi) disruption of the body boundary membrane. Autophagy was not involved in mitoptosis. Increased production of reactive oxygen species (ROS) was necessary for execution of the program, since antioxidants prevent mitoptosis and kill the cells treated with the mitochondrial poisons. Mitoptosis served for protection of the cells under the conditions of severe damage of mitochondria. It is suggested that exocytosis of mitoptotic bodies may be involved in maturation of reticulocytes and precursors of lens fiber cells.

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(S10) Mitochondria and reactive oxygen containing species symposium lecture abstracts

S10/1 Interactions of nitric oxide with cytochrome *c* and cytochrome *c* oxidase

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At low nM concentration nitric oxide (NO) is an intercellular messenger, interacting with the heme protein guanylate cyclase.