

### 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*<sup>-/-</sup> 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, eIF2 $\alpha$ , 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 mitofusins, 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,