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| Abstract | Inherited retinal dystrophies (IRDs) are a broad group of neurodegenerative disorders associated with reduced or deteriorating visual system. In the retina, cells are under constant oxidative stress, leading to elevated reactive oxygen species (ROS) generation that induces mitochondrial dysfunction and alteration of the mitochondrial network. This mitochondrial dysfunction combined with mutations in mitochondrial DNA and nuclear genes makes photoreceptors and retinal ganglion cells more susceptible to cell death. In this minireview, we focus on mitochondrial dynamics and their contribution to neuronal degeneration underlying IRDs, with particular attention to Leber hereditary optic neuropathy (LHON) and autosomal dominant optic atrophy (DOA), and propose targeting cell resilience and mitochondrial dynamics modulators as potential therapeutic approaches for retinal disorders. |

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| Keywords (separated by " - ") | Retinal dystrophies - Oxidative stress - Mitochondrial dynamics - Retinopathies - Neurodegeneration |

# 84 Mitochondrial Gymnastics in Retinal Cells: A Resilience Mechanism Against Oxidative Stress and Neurodegeneration

Serena Mirra and Gemma Marfany

**Abstract**

Inherited retinal dystrophies (IRDs) are a broad group of neurodegenerative disorders associated with reduced or deteriorating visual system. In the retina, cells are under constant oxidative stress, leading to elevated reactive oxygen species (ROS) generation that induces mitochondrial dysfunction and alteration of the mitochondrial network. This mitochondrial dysfunction combined with mutations in mitochondrial DNA and nuclear genes makes photoreceptors and retinal ganglion cells more susceptible to cell death. In this minireview, we focus on mitochondrial dynamics and their contribution to neuronal degeneration underlying IRDs, with particular attention to Leber hereditary optic neuropathy (LHON) and autosomal dominant optic atrophy (DOA), and propose targeting cell resilience and mitochondrial dynamics modulators as potential therapeutic approaches for retinal disorders.

**Keywords**

Retinal dystrophies · Oxidative stress · Mitochondrial dynamics · Retinopathies · Neurodegeneration

## 84.1 IRDs and Mitochondria

IRDs are a large group of diseases characterized by retinal cell degeneration and death. Among retinal cells, photoreceptors and ganglion cells receive the stressful impact of light photons and excess intraocular pressure; thus resilience mechanisms have to be switched on to promote cell survival. Correct mitochondrial metabolism and dynamics are essential for retinal cells and mutations in either mtDNA or in nuclear genes involved in mitochondrial function having a high impact on cell survival. Since progressive attrition of photoreceptors and retinal ganglion cells leads to blindness, the correct preservation of mitochondrial function and dynamics is essential for retinal homeostasis.

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Mitochondria are essential organelles that supply energy to the cell through oxidative phosphorylation (OXPHOS) and are also essential in calcium buffering, cell cycle control and regulation of apoptosis. Mitochondrial activity generates 1–5% ROS in physiological conditions (Nissanka and Moraes 2018). Severe alterations in mitochondrial function due to physiological and environmental cues generate loss of mitochondrial membrane potential ($\Delta\Psi m$), decreased OXPHOS, mitochondrial DNA (mtDNA) damage and ROS-induced ROS vicious circle (Bae et al. 2011). These changes are associated to remodelling of the mitochondrial network, and these mitochondrial dynamics include fusion, fission, transport, interorganellar communication and mitochondrial quality control (Cid-Castro et al. 2018). Although numerous studies have implicated ROS in neuronal death, their role in the pathophysiology of optical neuropathies remains to be further investigated. Leber hereditary optic neuropathy (LHON) and dominant optic atrophy (DOA) are two prototypic inherited ocular disorders related to mitochondrial dysfunction. Despite their contrasting genetic basis, they share overlapping pathological features due to the particular vulnerability of retinal ganglion cells (RGCs) to perturbed mitochondrial function.

## 84.2 Mitochondrial Localization within the Retina

The retina is among the most metabolically active tissues in the body due to its high oxygen demand. Allocation of mitochondria in high energy requirements regions is essential for cellular function. In the outermost layer of the vertebrate retina, rod and cone photoreceptors display highly differentiated outer segments, packed with membranous disks/folds where photoreception and the phototransduction cascade occur. The most distal tips of rod and cone outer segments closely interact with the retinal pigment epithelium (RPE), which continually supports photoreceptor function by endorsing the outer segments feeding and renewal. To meet the high demand of energy, photoreceptor cells display a high concentration of mitochondria in the outer part of the inner segment, whereas in RPE cells mitochondria are located at the basal region. Significantly, in the mammalian inner retina, mitochondria are particularly concentrated in the unmyelinated proximal axons of RGC compared to myelinated segment of the optic nerve. This particular distribution is required to guarantee the energy supply for the generation of an action potential that continuously propagates along these axonal regions. This high demand of energy together with a complex dendritic arborization underlies RGC susceptibility to respiratory chain dysfunction, oxidative stress and, eventually, apoptosis (Ito and Di Polo 2017).

## 84.3 Mitochondrial Dynamics: Fusion and Fission

Mitochondrial fusion requires the apposition of two adjacent organelles, followed by outer and inner membrane fusion. Fusion is mediated by the conserved dynamin-related GTP proteins mitofusins (Mfn1 and Mfn2) and the optic dominant atrophy 1 protein (OPA1). Mfns are distributed evenly on the outer mitochondrial membrane. Loss of Mfns impairs mitochondrial fusion, and consequently, mitochondrial length is reduced. OPA1 is anchored to the mitochondrial inner membrane and interacts with Mfns to form protein complexes that couple the fusion between the outer and inner membranes. *Opa1*-deficient mice display RGC mitochondrial fragmentation, dendritic atrophy prior to visual impairment and neuronal loss (Williams et al. 2010). Mitochondrial elongation confers resistance to apoptotic stimuli, and a network of fused mitochondria has been described in many senescent postmitotic cell types. How does fusion protect mitochondrial function? Mitochondrial fusion contributes to mitochondrial homeostasis by enabling the exchange of mtDNA, substrates, metabolites or specific lipids contents between organelles (Hoitzing et al. 2015).

Mitochondrial fission instead is mediated by the adaptor FIS1 protein and the dynamin-related

GTPase DRP1. FIS1 is spread diffusely throughout the outer mitochondrial membrane and recruits DRP1 from the cytosol. DRP1 assembles into spiral filaments around mitochondria tubules, constricting mitochondria through conformational changes. Interestingly, the tubules of the endoplasmic reticulum (ER) wrap around and squeeze mitochondria at the early stage of division, facilitating DRP1 recruitment to complete fission (Friedman et al. 2011). Of note, an excess of mitochondrial fission can represent the first step of apoptosis. Following extensive cellular stress or damage, the pro-apoptotic Bcl-2 family member Bax translocates to mitochondria and accumulates in concentrated foci that colocalize with DRP1 and Mfns. This process mediates pore formation in outer mitochondrial membranes, which facilitates the release of cytochrome C from mitochondria and downstream caspase activation.

In vivo models of retinal detachment showed that DRP1 activation can be induced by exogenous ROS, triggering mitochondrial fission previous to apoptotic cascade activation. Moreover, DRP1 inhibition results in a neuroprotective effect by suppressing mitochondrial fission and apoptosis (She et al. 2018). Consistently, mitochondrial stress induces mitochondrial fragmentation by increasing DRP1 in the retina of glaucomatous D2 mice and in cultured RGC in vitro. And increase in OPA1 expression and DRP1 inhibition blocks mitochondrial fission, with a subsequent reduction of oxidative stress and an increase of RGC survival (Ju et al. 2010; Kim et al. 2015).

## 84.4 Mitophagy: Mitochondria as the Main Course

Mitochondria have multiple quality control mechanisms to ensure mitochondrial integrity, and alterations in this quality control have been extensively associated to neurodegenerative diseases (Pickles et al. 2018). Fission sequesters irreversibly damaged or fusion-incompetent mitochondria and results in their subsequent elimination by mitophagy, the autophagy-mediated degradation of mitochondria (Shutt et al. 2012). The best-studied mitophagy pathway involves PINK1 and Parkin, genes associated to rare genetic forms of Parkinson's disease (McWilliams and Muqit 2017). When mitochondria lose their membrane potential, the mitochondrial protein PINK1 recruits the E3 ubiquitin ligase Parkin from the cytosol to dysfunctional mitochondria, where it ubiquitinates mitochondrial proteins for proteasomal degradation and promotes the engulfment of mitochondria by autophagosomes (Lazarou et al. 2015). These studies on PINK1/Parkin-dependent mitophagy were performed in vitro, and contribution of this pathway in vivo is yet to be determined.

Parkin is widely expressed in the murine retina, particularly in the RGCs (Esteve-Rudd et al. 2010). Conversely, PINK1 protein expression in the whole retina is very low, suggesting that retinal basal mitophagy occurs independently of PINK1 (McWilliams et al. 2018). Parkin overexpression stabilizes mitochondrial membrane potential and decreases glutamate cytotoxicity and apoptosis in RGCs (Hu et al. 2017). Also, Parkin expression is upregulated in murine model of hypertensive glaucoma, where its overexpression partially restores dysfunction of mitophagy in RGCs (Dai et al. 2018). All together these data point out the protective role of Parkin-mediated mitophagy against several damages potentially leading to optic neurodegeneration.

## 84.5 Mitochondrial Transport

As mentioned, mitochondrial transport and distribution to regions with high energy demand is crucial in neurons. The kinesin superfamily proteins (KIFs) and cytoplasmic dynein are the main motor proteins that transport mitochondria towards the microtubule positive and negative ends, respectively. In mammals, kinesin contacts the molecular adaptors Trak1 and Trak2, which in turn bind the GTPases Miro1 and Miro2 (Lopez-Domenech et al. 2018). Miro and Trak proteins interact with the machinery involved in fusion and fission, thus connecting mitochondrial dynamics and trafficking processes. Moreover,

the PINK/Parkin complex targets Miro for degradation, and as a consequence, kinesin is released from mitochondria, thus leading to a redistribution of damaged mitochondria (Wang et al. 2011).

In contrast to mature dendrites, mitochondria are highly dynamic in RGC axons. In early stages of a glaucoma mice model, the number of transported mitochondria in RGC decreased, and axons were devoid of mitochondria before RGC death (Takihara et al. 2015). Several evidences highlight the importance of cytoskeleton integrity for the correct mitochondrial motility along RGC processes (Tang 2018). Moreover, new players regulating mitochondrial trafficking in neurons have been recently described. Among them, the Miro-interacting mitochondrial protein Armcx1 enhanced mitochondrial transport in adult RGC and promoted axonal regeneration after injury (Cartoni et al. 2016). Nonetheless, the molecular role of motor and adaptor proteins mediating mitochondrial transport in RGC remains to be further elucidated.

## 84.6 Mitochondrial Optic Neuropathies

Primary mitochondrial disorders (PMD) are associated to pathogenic mtDNA or nuclear gene mutations, whereas secondary mitochondrial disorders (SMD) are mainly due to nongenetic causes, e.g. environmental factors or pharmacological toxins. In Leber hereditary optic neuropathy (LHON), several mtDNA mutations lead to dysfunction in the mitochondrial complex I, causing accumulation of ROS and cell death in the RGC cells. About 90% of LHON cases are caused by point mutation in MT-ND1, MT-ND4, MT-ND4L or MT-ND6 genes (Kim et al. 2018), but it remains unclear how these genetic alterations lead to the specific features of LHON.

DOA is an autosomal dominant PMD characterized by progressive blindness with degeneration of RGC and the optic nerve, with a prevalence of 1:35,000 people worldwide. Approximately 50–60% of DOA patients carry mutations in the nuclear OPA1 gene, which regulates mitochondria fusion and OXPHOS and is involved in cell death and mtDNA maintenance. A different set of OPA1 mutations causes "DOA-plus" phenotypes with mtDNA instability, deafness and movement disorders in addition to traditional DOA symptoms (Pilz et al. 2017).

Other mitochondrial dysfunction syndromes with marked optic neuropathy are Charcot-Marie-Tooth disease (when caused by MFN2 mutations), Friedreich Ataxia and Costeff syndrome, although the mechanisms that trigger RGC death in the two latter are far from understood (Carelli et al. 2017).

## 84.7 Future Perspectives

Several therapeutic strategies have been tested over the years to prevent mitochondrial dysfunction-related neurodegeneration. Modification of ROS production or inhibition of caspase apoptotic pathway has been both employed in clinical trials, but these strategies failed to prevent neurodegeneration.

Targeting mitochondrial dynamics means to intervene between the triggering event (ROS generation) and the terminal phase (cell death); thus it may represent an effective approach to prevent progressive degeneration. Screening compounds targeting the mitochondrial fission/fusion machinery and the mitochondrial quality control system would impact in the recovery of a "healthy" mitochondrial network and, as a consequence, improve the neurological phenotype. However, until now, no novel therapeutic strategy specifically targeting mitochondrial dynamics has been developed.

On the other hand, retinal cells deploy several pathways to deal with oxidative stress, which are most likely interconnected. Studying IRD genes that encode key protein sensors and modulators that play a role in the crosstalk between theformation of lipid droplets (e.g. *MTTP*, *TTPA*, *CLN3*, *PNPLA6*), mRNA stress granules (e.g. *CERKL*), mitochondrial dynamics (e.g. *MFN2*, *SLC25A46*, *OPA3*, *OPA8*) and autophagy (e.g. *DRAM2*) will accrue knowledge on survival versus apoptosis fate decisions in retinal cells and may offer new scenarios for therapeutic targets.

We propose that future drug and gene therapies addressed to reduce mitochondrial fission, increase mitochondrial fusion and favour other resilience cell mechanisms would favour retinal cell survival, preventing or halting the progression of the retinal degeneration.

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