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A localized autophagic filter prevents entry of mitochondria carrying pathogenic Opa1 mutations in retinal ganglion cell axons

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1. Riassunto dell'attività svolta

I mitocondri sono organelli estremamente dinamici, che sono dotati di un complesso apparato per fusione e fissione. La fissione viene regolata da Dynamin related protein 1 (DRP1), che trasloca ai mitocondri in seguito a defosforilazione mediata da calcineurina (Yoon et al., 2001; Smirnova et al., 2001a; Cereghetti et al., 2008a). Le due Mitofusine presiedono la fusione della membrana mitocondriale esterna (Santel and Fuller, 2001a; Legros et al., 2002a; Chen et al., 2003a; Santel et al., 2003a), mentre Optic Atrophy 1 (OPA1), una proteina appartenente alla famiglia delle dinamine, coopera con MFN1 per la fusione della membrana mitocondriale interna (Cipolat et al., 2004b). OPA1 ha il ruolo chiave nel regolare la forma delle *cristae*, strutture pleomorfe della membrana mitocondriale interna. In particolare, OPA1 è localizzata a livello delle *cristae junctions*, formando oligomeri che regolano l'apertura delle *cristae*, e quindi il citocromo c, un fattore solubile che regola l'apoptosi (Cipolat et al., 2006b; Frezza et al., 2006a). Recentemente è stato dimostrato che OPA1 ha un ruolo addizionale nell'assemblaggio dei supercomplessi della catena respiratoria attraverso la modulazione dell'ultrastruttura della membrana mitocondriale interna (Cogliati et al., 2013).

Opa1 è mutata nell'atrofia ottica dominante (ADOA), la neuropatia ottica ereditaria più diffusa, dovuta alla degenerazione selettiva dei neuroni gangliari della retina (RGC). La fisiopatologia dell'ADOA non è ancora nota. Osservazioni da studi *in vitro* su colture neuronali mostrano alterazioni mitocondriali morfologiche e funzionali: i mitocondri sono frammentati, impropriamente aggregati nel soma e con ridotta capacità di fare *buffer* di calcio (Kamei et al., 2005; Dayanithi et al., 2010; Bertholet et al., 2013; Kushnareva et al., 2013).

Nei neuroni, i mitocondri vengono trasportati lungo i microtubuli in siti con alta richiesta energetica, come i nodi di Ranvier e le sinapsi, dove fanno *buffer* di calcio e generano ATP per sostenere l'attività neuronale (Sheng and Cai, 2012; Itoh et al., 2013).

Inoltre, molte patologie neurodegenerative, quali Alzheimer, Parkinson e Sclerosi Laterale Amiotrofica, presentano una forte relazione tra mitocondri disfunzionali e autofagia (Schapira et al., 1990; Betarbet et al., 2000; Nixon et al., 2005; Sasaki et al., 2005; Magrane et al., 2009; Yao et al., 2009; Chinta et al., 2010). La degradazione di mitocondri non funzionali è un processo chiamato mitofagia. I mitocondri frammentati e stazionari sono più propensi ad essere circondati da una vescicola con doppia membrana,

l'autofagosoma, e successivamente degradati (Twig et al., 2010). La depolarizzazione dei mitocondri induce l'attivazione della Pink1/Parkin *pathway* (Greene et al., 2003; Park et al., 2006; Narendra et al., 2008; Matsui et al., 2013), che poliubiquitina proteine della membrana mitocondriale esterna che indirizza i mitocondri all'autofagia (Gegg et al., 2010; Geisler et al., 2010; Ziviani et al., 2010a; Chan et al., 2011). Nel 2007, Davies e collaboratori hanno dimostrato un ruolo centrale dell'autofagia nell'ADOA. In particolare, analisi ultrastrutturali hanno mostrato l'aumento di vescicole autofagiche prima dello sviluppo di atrofia ottica in un modello murino di ADOA (Davies et al., 2007; White et al., 2009). I RGC sono il modello più adeguato per lo studio dell'ADOA. Questi neuroni presentano mitocondri particolarmente concentrati nel segmento prossimale dell'assone, che richiede molta energia per sostenere i potenziali d'azione (Carelli et al., 2004). L'inattivazione di OPA1 potrebbe essere particolarmente importante in RGC, che hanno bassa attività glicolitica e dipendono quasi esclusivamente dall'energia fornita dai mitocondri. Pertanto, lo scopo di questa tesi era di studiare l'effetto di mitocondri non funzionali portatori di mutanti patogenici di Opa1 in colture primarie di RGC. In questo modello, l'espressione di mutanti patogenici di Opa1 causa diverse disfunzionalità mitocondriali, come frammentazione, depolarizzazione e immobilità. Questo fenotipo è caratteristico di mitocondri destinati all'autofagia. Dati di *live imaging* mostrano che i mitocondri si accumulano in prossimità dell'ilo assonale, dove vengono degradati mediante autofagia, in questo modo impedendo il loro ingresso negli assoni. Quindi, abbiamo modulato l'autofagia usando diversi inibitori e quando l'autofagia è inibita, i mitocondri esprimenti mutanti patogenici di Opa1 si ridistribuiscono negli assoni. Inoltre, i RGC sono meno sensibili a induttori di apoptosi. Questo fenotipo potrebbe essere dovuto a due diversi meccanismi concomitanti. Osservazioni farmacologiche e genetiche sostengono un ruolo per meccanismi mediati da Ca^{2+} -calcineurin e AMP/AMPK nell'accumulo di mitocondri nell'ilo assonale e loro degradazione autofagica. Inoltre, la sensibilità all'apoptosi dei RGC viene ridotta dall'inattivazione di calcineurina. In conclusione, noi abbiamo dimostrato che l'inattivazione di Opa1 induce un'autofagia disregolata in prossimità all'ilo assonale, dove i mitocondri non funzionali vengono attivamente degradati, e questo meccanismo potrebbe essere fondamentale nella patogenesi dell'ADOA.

2. Summary

Mitochondria are dynamic organelles that are endowed by a complex fission/fusion machinery. Fission is regulated by the Dynamin related protein 1 (DRP1), which translocates to mitochondria by calcineurin dependent dephosphorylation (Yoon et al., 2001; Smirnova et al., 2001a; Cereghetti et al., 2008a). The two Mitofusins orchestrate the fusion of the outer mitochondrial membrane (Santel and Fuller, 2001a; Legros et al., 2002a; Chen et al., 2003a; Santel et al., 2003a), while Optic Atrophy 1 (OPA1), a dynamin related-protein, cooperates with MFN1 in fusion of the inner mitochondrial membrane (Cipolat et al., 2004b). OPA1 has a key role in regulating the shape of mitochondrial *cristae*, pleomorphic structures of the inner mitochondrial membrane. In particular, OPA1 is localized at the level of *cristae* junctions, forming oligomers that regulate the opening of the *cristae* and thus, the release of cytochrome *c*, a soluble factor involved in apoptosis (Cipolat et al., 2006b; Frezza et al., 2006a). Recently, it has been shown that OPA1 has an additional role in the assembly of respiratory chain supercomplexes by modulating the ultrastructure of the inner mitochondrial membrane (Cogliati et al., 2013).

Opa1 is mutated in autosomal dominant optic atrophy (ADOA), the most common of inherited optic neuropathy, caused by the selective loss of retinal ganglion cells (RGCs). The pathophysiology of ADOA is still unknown. Evidences from *in vitro* studies on neuronal cultures reveal mitochondrial abnormalities in morphology and function: mitochondria appear fragmented, improperly accumulated in the soma, and with impaired potential and Ca^{2+} buffer capacity (Kamei et al., 2005; Dayanithi et al., 2010; Bertholet et al., 2013; Kushnareva et al., 2013).

In neurons, mitochondria are transported along microtubules in sites of high energy demand as Ranvier Nodes and synapses, where they buffer Ca^{2+} and produce ATP to sustain neuronal activity (Sheng and Cai, 2012; Itoh et al., 2013).

Moreover, many neurodegenerative diseases, as Alzheimer's disease, Parkinson's disease and Amyotrophic Lateral Sclerosis, present a strong relationship between dysfunctional mitochondrial and autophagy (Schapira et al., 1990; Betarbet et al., 2000; Nixon et al., 2005; Sasaki et al., 2005; Magrane et al., 2009; Yao et al., 2009; Chinta et al., 2010). The degradation of dysfunctional mitochondria through autophagy is a process called mitophagy. Fragmented and stationary mitochondria are easily engulfed by a double-membrane vesicle, the autophagosome, and subsequently degraded (Twig et al.,

2010). Depolarization of mitochondria triggers the activation of the Pink1/Parkin pathway (Greene et al., 2003; Park et al., 2006; Narendra et al., 2008; Matsui et al., 2013), which add ubiquitin chains on proteins of the outer mitochondria membrane targeting mitochondria to the autophagosome (Gegg et al., 2010; Geisler et al., 2010; Ziviani et al., 2010a; Chan et al., 2011).

In 2007, Davies and colleagues have demonstrated a central role of autophagy in ADOA. In particular, ultrastructural analysis revealed increased autophagic vesicles preceding optic atrophy in an ADOA mouse model (Davies et al., 2007; White et al., 2009). RGCs are the adequate model to study ADOA pathology. These neurons are enriched in mitochondria in the proximal segment of the axon, that has high energy demand to sustain action potentials (Carelli et al., 2004). OPA1 inactivation could be particularly important in RGCs, which have a poor glycolytic activity and thus depend almost exclusively on the energy provided by mitochondria. Therefore, the aim of this thesis was to study the impact of dysfunctional mitochondria carrying Opa1 pathogenic mutants in primary RGCs.

In this model, the expression of Opa1 mutants caused mitochondrial dysfunction, including fragmentation, depolarization and immobility. Indeed, this phenotype is representative of mitochondria targeted to autophagy. Live imaging data revealed that mitochondria accumulated in proximity of the axon, where they were actively degraded by autophagy that prevented their entry into the axons. Therefore, we modulated autophagy using different inhibitors and when autophagy was blocked, mitochondria expressing pathogenic Opa1 mutants redistributed in axons. Moreover, RGCs were less sensitive to apoptotic inducers. This phenotype could be due by two different/concomitant mechanisms. Pharmacological and genetic evidences support both a role for a Ca^{2+} -calcineurin and one for AMP/AMPK in mitochondrial accumulation at the axonal hillock and degradation by autophagy. Interestingly, blockage of calcineurin reduced RGCs sensitivity to apoptosis. In conclusion, we demonstrated that OPA1 dysfunction induces abnormal autophagy close to the axonal hillock, where dysfunctional mitochondria are actively degraded, and this mechanism could be fundamental in the pathogenesis of ADOA.

3. Introduction

3.1. Mitochondria

Mitochondria are double membrane cellular organelles which contain about one tenth of all cellular proteins. On a weight basis proportion, it has been calculated that mitochondria can convert between 10.000 and 50.000 times per second more energy than the sun (Schatz, 2007; Bolanos et al., 2010).

The establishment of a technique to isolate mitochondria (Claude, 1946) and the discovery of the biochemical properties of mitochondria, that is, the presence of a respiratory system, the tricarboxylic acid cycle and fatty acid oxidation (Kennedy and Lehninger, 1949), permitted the start of the so called bioenergetics era. This period peaked with Peter Mitchell's chemiosmotic theory (Mitchell and Moyle, 1965), that deserved him the Nobel Prize for chemistry in 1978. According to his theory, the free energy of respiration is used to pump protons (H^+) from the matrix to the inter-membrane space (IMS), generating an electrochemical gradient. It is defined as the sum of the proton concentration difference and the electrical potential difference across the membrane. An electrochemical gradient ($\Delta\mu_{H^+}$) builds-up across the membrane because the inner mitochondrial membrane (IMM) is characterized by an extremely low passive permeability to H^+ . In normal conditions, the majority of the gradient is constituted by the electrical potential difference, which has been estimated to measure about -220 mV (negative inside). The F_1F_0 -ATP synthase converts the proton gradient in ATP, combining the reversal transport of H^+ in the matrix with the phosphorylation of ADP to ATP.

3.2. Mitochondria ultrastructure

Studies from Palade and Sjostrand revealed that mitochondria present two membranes – an outer mitochondrial membrane (OMM) and a highly convoluted IMM, folded in a series of ridges that were named *cristae* by Palade (Palade, 1952; SJOSTRAND, 1953). In the classical model (the baffle models) described by Palade and still reported in many text

books, *cristae* are considered invaginations of the IMM with broad openings to the IMS (Fig. 1A).

The improvements in electron microscopy allowed researchers to better investigate mitochondrial ultra-structure. The electron tomography applied to mitochondria revealed that *cristae* are not simple invaginations of the IMM, but they represent a distinct compartment that connects to the IMS by narrow tubular connections, called *cristae* junctions (Mannella et al., 1994; Perkins et al., 1997). The average diameter of *cristae* junctions is 28 nm and the distance across the OMM and IMM is about 20 nm (Fig.1B).

Cristae junctions act as a functional barrier between the *cristae* space and the IMS (Fig. 1C,D). The presence a distinct compartment has important functional consequences because it suggests a limited diffusion between *cristae* and IMS. Oxidative phosphorylation takes place on *cristae* (Gilkerson et al., 2003). Accordingly, *cristae* are equipped with oxidative phosphorylation proteins and ATP synthase dimers are assembled at tips of the *cristae* (Giraud et al., 2002; Strauss et al., 2008). This highly defined compartmentalization suggests *cristae* as optimal niches for ATP production because they block the passive diffusion of important players of respiration, such as H⁺ or ADP (Demongeot et al., 2007). Moreover, the respiratory condition of mitochondria alters shape and density of the *cristae* (Hackenbrock, 1968; Hackenbrock et al., 1980). It is intriguing to note that the majority of cytochrome *c* is also localized into the *cristae* (Scorrano et al., 2002) and it may suggest that *cristae* have a regulatory role not only in respiration but also apoptosis, when cytochrome *c* is released from mitochondria to the cytoplasm.

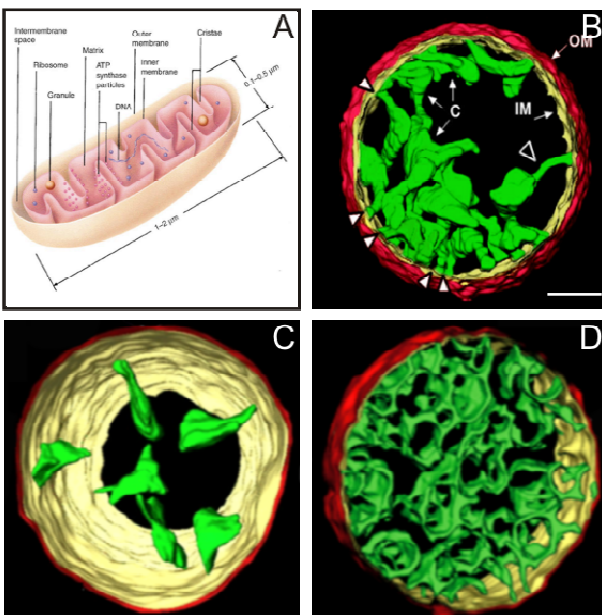


Fig.1. Mitochondrial ultrastructure. (A) A text book-like representation of the baffle model adapted from (Frey and Mannella, 2000). (B) Three-dimensional reconstructions of isolated rat liver mitochondria obtained by high-voltage electron tomography. OM: outer membrane, IM: inner membrane, C: selected cristae; arrowheads point to narrow tubular regions that connect cristae to periphery and to each other. Bar, 0.4 μm. Adapted from (Frey and Mannella, 2000). (C-D) Representative surface-rendered views of electron microscopy tomography reconstructions of mitochondria before (C) and after (D) remodelling. The OM is depicted in red, the inner boundary membrane in yellow, and the cristae in green (Scorrano et al., 2002).

3.3. Mitochondrial dynamics

Mitochondria undergo cycles of fusion and fission and change their shape continuously, assuming the form of spheres and interconnected tubules (Bereiter-Hahn and Voth, 1994). Two mitochondria may come in contact and fuse (Bereiter-Hahn and Voth, 1994; Chen et al., 2003b) but mitochondria can also divide, giving rise to two or more daughter mitochondria. A transient form of fusion, called "kiss-and-run", was recently described; two mitochondria contact each other, exchange soluble and matrix components, and separate without changing morphology (Liu et al., 2009). Mitochondria are equipped by two membranes and during fusion and fission cycles, the fusion and division of four lipid bilayers must be finely regulated. Thus, fusion and fission events are controlled by a growing number of mitochondrial-shaping proteins.

3.3.1 Fusion machinery

Mitochondrial fusion is a mechanism that is thought to be conserved from lower to higher eukaryotes. An intact membrane potential is a prerequisite for mitochondrial fusion, which is independent of a functional cytoskeleton (Legros et al., 2002b; Mattenberger et al., 2003). In yeast, the fusion of outer mitochondrial membranes (OMMs) requires homotypic trans-interactions of the Fzo1, the proton gradient component of the inner membrane electrical potential, and low levels of GTP hydrolysis. Fusion of inner mitochondrial membranes (IMMs) requires the electrical component of the inner membrane potential and high levels of GTP hydrolysis. Time-lapse analysis of mitochondrial fusion in yeast and mammalian cells, *in vivo*, clearly shows that fusion of the OMM and IMM are temporally linked. These observations indicate that individual fusion machineries exist in each membrane, but they can communicate *in vivo*, resulting in coupled outer and inner membrane fusion (Meeusen et al., 2004). The major components and regulators of the fusion machinery will be discussed below.

The *Drosophila* Fuzzy onions 1 protein (Fzo1) is a large transmembrane guanosine triphosphatase (GTPase) essential for the genesis of the giant mitochondrial derivative during spermatogenesis (Hales and Fuller, 1997). The *S. Cerevisiae* ortholog of Fzo1 induces mitochondrial fusion during mitotic growth and mating (Hermann et al., 1998). Mitofusin (MFN) 1 and 2 are Fzo1 mammalian homologues (Eura et al., 2003; Rojo et al.,

2002; Santel et al., 2003b) which conserve the GTPase domain, even if MFN1 shows a higher GTPase activity than MFN2 (Ishihara et al., 2004). *Drosophila* presents another MFN homologue, the mitochondrial assembly regulatory factor (Marf) (Deng et al., 2008). MFN1 and 2 have the 81% of identity, similar topologies and localization in the inner mitochondrial membrane (Chen et al., 2003b; Legros et al., 2002b; Rojo et al., 2002; Santel and Fuller, 2001b; Santel et al., 2003b). Both present a cytosolic coiled coil motif (Koshiba et al., 2004; Rojo et al., 2002; Santel, 2006) which is necessary for protein-protein oligomerization (Oakley and Hollenbeck, 2001). Thus, two MFNs on opposing membranes can bind in *trans* to bridge mitochondria (Koshiba et al., 2004). However, *Mfn1*^{-/-} and *Mfn2*^{-/-} cells show different mitochondrial fusion rates; resulting in higher fusion events in cells containing only MFN1 (Chen et al., 2003b). Finally, MFN1, but not MFN2, is essential for OPA1-dependent mitochondrial fusion in embryonic fibroblasts (Cipolat et al., 2004a). In addition, MFN2 is very abundant at the level of the mitochondria-ER interface and present (albeit to a lesser extent) at the ER, where it tethers mitochondria to ER and regulates ER shape. As a regulator of the ER-mitochondria interaction, MFN2 generates Ca²⁺ micro-domains between ER and mitochondria and, thus, regulates Ca²⁺ uptake (de Brito and Scorrano, 2008a). Several regulators of MFNs activity have been characterized. Mitofusin-binding protein interacts with MFN1 and causes mitochondrial fragmentation (Eura et al., 2006). MFN1 and MFN2 expression levels have been reported to increase upon inhibition of the proteasome, suggesting its involvement in MFNs degradation (Karbowski et al., 2007). PARKIN, an E3-ubiquitin ligase discussed below, ubiquitinates MFN1, MFN2 (Gegg et al., 2010; Tanaka et al., 2010a) and MARF (Ziviani et al., 2010b), targeting them to proteasomal degradation (Tanaka et al., 2010a). BAX and BAK have been found in a high-molecular weight complexes with MFN2, which inhibit fusion by altering the assembly, mobility and distribution of MFN2 complexes (Karbowski et al., 2006).

Optic atrophy 1 (OPA1) is a dynamin-related protein located in the IMM which has been identified by two independent groups (Alexander et al., 2000b; Delettre et al., 2000b). OPA1 has two yeast homologues, Mgm1 (Jones and Fangman, 1992) and Msp1 (Pelloquin et al., 1998). Mgm1, Msp1 and OPA1 are localized in the intermembrane space (IMS), tightly associated with the IMM (Guillou et al., 2005; Herlan et al., 2003; Olichon et al., 2002; Sesaki et al., 2003; Wong et al., 2003). These proteins display 20% of sequence identity but show a highly conserved secondary structure, consisting of two coiled coils, one amino-terminal to the GTPase domain and the other at the carboxy-terminus. The

carboxy-terminal coiled coil domain of OPA1 may function as a GTPase effector domain (GED). On its amino-terminal, OPA1 possesses a mitochondrial targeting sequence which is cleaved by the mitochondrial processing peptidase upon import in mitochondria (Sato et al., 2003). Mgm1 and Msp1 maintain fusion events in yeast and Mgm1 complexes with Fzo1 to coordinate the fusion of the IMM and OMM (Wong et al., 2003). Overexpression of OPA1 or its downregulation by siRNA in mouse embryonic fibroblasts (MEFs) discloses a linear relationship between OPA1 levels and mitochondrial fusion (Cipolat et al., 2004a). Interestingly, OPA1/Mgm1 controls cristae structure and remodelling (Griparic et al., 2004a; Olichon et al., 2003a; Sesaki et al., 2003). Our laboratory demonstrated that OPA1 can regulate cytochrome *c* mobilization and apoptotic *cristae* remodelling independently of its pro-fusion activity; OPA1 organizes into high molecular weight complexes, that control *cristae* morphology and are targeted by BID during apoptosis (Frezza et al., 2006b). Also Mgm1 maintains *cristae* structures through Mgm1 interactions on opposing inner membranes (Meeusen et al., 2006). In human, OPA1 is present in 8 splicing isoforms (Delettre et al., 2001), which can be post-translationally cleaved in two sites, S1 and S2, resulting in five bands on a western-blot. The two higher molecular weight bands represent proteins integrated into the IMM, whereas the three lower molecular weight bands lack the transmembrane domain and are localized in the IMS (Duvezin-Caubet et al., 2007). In particular, it seems that mainly long forms support mitochondrial fusion (Song et al., 2007). Different proteases regulates OPA1 processing. The ATP-dependent matrix AAA (m-AAA) protease paraplegin, when the membrane potential is dissipated, stimulates OPA1 processing and mitochondrial fragmentation (Ishihara et al., 2006). OMA1 is an ATP-independent peptidase in the IMM, which mediates OPA1 processing in absence of m-AAA proteases or impairment of mitochondrial activity (Ehres et al., 2009). Mitochondrial membrane potential modulates the i-AAA protease YME1L (Guillery et al., 2008). Prohibitins processes long forms of OPA1 and their ablation causes fragmentation and aberrant *cristae* (Merkwirth et al., 2008). Finally, PARL is involved in OPA1 processing, resulting in the generation of a soluble form of OPA1 localized in the IMS (Cipolat et al., 2006a). Regulation of OPA1 processing by PARL regulates exclusively its role in apoptosis (Frezza et al., 2006b). Higd-1a binds and inhibits Opa1 cleavage and trough its interaction with OPA1 is required for mitochondrial fusion (Hyun-Jung et al., 2013). Another regulator of fusion activity is MitoPLD. It localizes to the OMM and hydrolyzes cardiolipin to form phosphatidic acid, which facilitates fusion driven by specialized SNARE-complexes (Choi et al., 2006).

3.3.2 Fission machinery

Dynamin-related protein 1 (DRP1) is a protein similar to dynamin, a large GTPase involved in membrane scission in multiple endocytic and secretory organelles (Praefcke and McMahon, 2004). The majority of DRP1 fraction is cytosolic but it could be also found in mitochondrial sites of constriction (Labrousse et al., 1999; Smirnova et al., 2001b). DRP1 *in vitro* oligomerizes in ring-like structures similar to those at membrane constriction sites. DRP1 regulates fission through GTP hydrolysis and mitochondrial membrane constriction (Hinshaw, 1999; Smirnova et al., 2001b). DRP1 can be phosphorylated at serine 637 by the cAMP-dependent protein kinase A (PKA) halting fission; whereas the phosphorylation at serine 637 by calcium/calmodulin-dependent protein kinase I alpha (CAMK1 α) (Han et al., 2008a) or at serine 616 by cyclin-dependent kinase 1 (CDK1) (Taguchi et al., 2007) causes mitochondrial fission. Conversely, DRP-1 Ca²⁺-dependent dephosphorylation by calcineurin at serine 637 induces translocation of DRP1 to mitochondria (Cereghetti et al., 2008b). DRP1 SUMOylation by mitochondrial-anchored protein ligase (MAPL) blocks ubiquitin attachment sites and thus inhibits proteasome degradation (Braschi et al., 2009). Conversely, the SUMO protease SENP5 deSUMOylates DRP1 and its ablation induces mitochondrial fragmentation (Zunino et al., 2007). MARCH5 is an OMM E3 ubiquitin ligase which ubiquitinates DRP1 (Nakamura et al., 2006; Yonashiro et al., 2006), thus regulating DRP1 localization and assembly at the scission sites on mitochondria (Karbowski et al., 2007). Moreover, overexpression of MTP18 causes DRP1-dependent mitochondrial fragmentation (Tondera et al., 2004; Tondera et al., 2005), even if its relation with DRP1 is still unclear.

FIS1 is an OMM protein present on the surface of mitochondria (James et al., 2003). Even if it does not show any enzymatic activity, FIS1 overexpression fragments mitochondria. Crosslinking and co-immunoprecipitation studies show the DRP1 and FIS1 interaction (Yoon et al., 2003). Thus, FIS1 probably recruits DRP1 to constriction sites on mitochondria. However, downregulation of FIS1 only partially diminishes DRP1 recruitment to mitochondria (Lee et al., 2004a) and it seems that other proteins are involved in the fission machinery. Indeed, mitochondrial fission factor (MFF) is an integral protein of OMM recruits DRP1 to the OMM independently of FIS1 (Otera et al., 2010). Another pro-fission candidate is LETM1, which fragments mitochondria independently of DRP1 (Dimmer et al., 2008).

3.4. Mitochondrial transport

Mitochondria are transported along microtubules in mammals and along actin filaments in yeast (Frederick and Shaw, 2007), even if an actin-based mechanism could regulate a subset of mitochondria movements, mostly localized in dendrites, nerve terminals, and growth cones (Chada and Hollenbeck, 2004). For instance, the disruption of the actin cytoskeleton in locust photoreceptors interrupts the photo-dependent movement of mitochondria (Sturmer et al., 1995). Myo19, Myosin V and Myosin VI are good candidates to mediate the actin-based transport of mitochondria (Quintero et al., 2009; Pathak et al., 2010).

The direction of the transport depends largely on the polarity and organization of neuronal microtubules. All axonal microtubules are oriented with their minus ends towards the soma and their plus ends towards the axon terminal, whereas microtubules in dendrites show mixed orientations. Mitochondrial transport in these compartments is mediated by two motor proteins. Kinesin superfamily proteins (KIFs) move towards the microtubule plus end and dynein mediates the minus end transport. Thus, in the axon KIFs and dynein regulate the anterograde (from the soma to the periphery) and retrograde (from the axon terminal to the soma) transport of cargoes, respectively. The KIF5 family mediates the transport of neuronal organelles, including mitochondria (Tanaka et al., 1998; Pilling et al., 2006). Mammalian KIF5s have three isoforms; KIF5B is abundantly expressed in different cell types, whereas KIF5A and KIF5C are neuronal specific (Kanai et al., 2000). Motor adaptors recognize and mediate the transport of mitochondria. The first studies on mitochondrial adaptors have been carried in *Drosophila* (Stowers et al., 2002). The motor adaptor Milton binds directly to the C-terminal cargo domain of KIF5 (Glater et al., 2006) and to mitochondrial rho (MIRO), a RHO family GTPase localized on the mitochondrial outer membrane (Frederick et al., 2004). Mutations of Milton or MIRO in *Drosophila* disrupts anterograde trafficking of mitochondria and depletes mitochondria from neurites (Stowers et al., 2002; Guo et al., 2005). There are two proposed models for the MIRO regulation of mitochondrial trafficking (Fig.2). MIRO contains two EF-hands motifs which bind Ca^{2+} . In neurons, Ca^{2+} varies according to synaptic activity or Ca^{2+} signalling pathways. In absence of Ca^{2+} , the C-terminal region of KIF5 binds the MIRO-Milton complex and the N-terminal region of KIF5 contacts microtubules, allowing the transport of the mitochondrion. In presence of Ca^{2+} , mitochondria arrest. According to the "motor-

MIRO binding" model (Wang and Schwarz, 2009b), Ca^{2+} binding by MIRO EF-hands induces the exposure of a KIF5 binding domain, which is sequestered from microtubules. In the "motor-releasing" model proposed by (MacAskill et al., 2009), Ca^{2+} induces the dissociation of KIF5 from the MIRO-Milton complex.

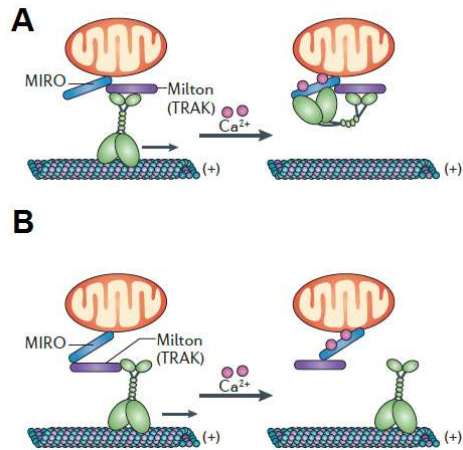


Fig. 2. The Miros/Milton transport of mitochondria. Two models of the KIF5 based mitochondrial transport have been described. **(A)** In the "motor-MIRO binding" model, Ca^{2+} binds to MIRO and induces the exposure of a KIF5 domain sequestered from microtubules. **(B)** In the "motor-releasing" model, KIF5 dissociates from the MIRO-Milton complex. From (Sheng and Cai, 2012).

However, these conclusions derive from MIRO EF-hand mutants which fail to stop mitochondria even at high Ca^{2+} concentrations. Interestingly, MIRO EF-hand mutants reduce the Ca^{2+} entry in mitochondria, which is correlated with the speed of mitochondrial movement in axons (Chang et al., 2011). Mitochondria buffer Ca^{2+} to maintain the homeostatic levels and in normal conditions, Ca^{2+} enters in matrix through the mitochondrial calcium uniporter, named MCU (Baughman et al., 2011; De et al., 2011). The blockage of MCU in high cytoplasmic Ca^{2+} do not alter mitochondrial motility (Chang et al., 2011). Thus, an intriguing hypothesis is to state mitochondrial Ca^{2+} as a determinant of mitochondrial transport (Niescier et al., 2013), even if the mechanism is not yet clear. In mammals, two orthologues of Milton (TRAK1 and TRAK2) and MIRO (MIRO1 and MIRO2) have been described. TRAKs bind the first GTPase domain of MIROs (Fransson et al., 2006). Interestingly, TRAK1 and TRAK2 mediates the transport of endosomes and neuronal proteins, for instance the GABA_A receptor and the K^+ channel Kir2.1 (Grishin et al., 2006; Webber et al., 2008; Stephenson, 2013). It has been shown that dynein interacts with Milton and MIRO and in Milton mutant flies the dynein-dependent movement of mitochondria is disrupted (Stowers et al., 2002). Further studies reported that TRAK1 binds kinesin and dynein but TRAK2 shows a preferential interaction with dynein, thus regulating mitochondrial trafficking in axon and dendrites (van et al., 2013). Another subset of mitochondrial adaptors is emerging from other studies. Syntabulin and FEZ1 are two novel interactors of KIF5. SiRNA downregulation of them reduces

anterograde transport of mitochondria in axons of hippocampal neurons (Cai et al., 2005; Fujita et al., 2007; Ikuta et al., 2007). Another candidate is RANBP2, which links mitochondria to KIF5B or KIF5C. Inhibition of RANBP2 induces the perinuclear clustering of mitochondria but its relevance in neurons is still not clear (Cho et al., 2007). Moreover, two other mitochondrial motors are emerging candidates for mitochondrial trafficking. KIF1B α and KLP6 belong to the kinesin motor family; mutants of both protein result in variations of the mitochondrial velocity in the axon (Tanaka et al., 2011).

The dynein binding to mitochondria is a field not very explored, since just few dynein heavy chains have been characterized. However, some studies identified VDAC1 and MIRO as possible dynein adaptors (Schwarzer et al., 2002; Russo et al., 2009).

Even if mitochondrial transport is essential for cell function and survival, only the 10% of mitochondria are in state of transport in hippocampal neurons. Among stationary mitochondria, the 40% of them conserves the same position for days (Obashi and Okabe, 2013). Thus, a special "stationary" apparatus is needed. Syntaphilin anchors mitochondria to microtubules (Kang et al., 2008) and this interaction is stabilized by LC8, a dynein light chain (Chen et al., 2009). Overexpression of syntaphilin or LC8 recruits mobile mitochondria in the stationary state; conversely, deletion of syntaphilin increases the percentage of mobile mitochondria. Microtubules-associated proteins (MAPs) and Tau are proteins which stabilize microtubules polymerization. MAP2 is localized in dendrites, whereas MAP1B and Tau are axon specific. Accumulation of Tau in neuroblastoma cell lines, cortical neurons, and retinal ganglion cells disrupts the kinesin-dependent transport of mitochondria. Tau association to microtubules reverses the direction of dynein transport, and kinesin fails to contact microtubules (Dixit et al., 2008). MAPB1 is also an inhibitor of the axonal retrograde transport of mitochondria (Jimenez-Mateos et al., 2006). Mitochondria can be docked also by the stability of the actin cytoskeleton, since WAVE1 regulates mitochondrial movement in dendritic spines in an actin polymerization-dependent mechanism, which is regulated by NMDA activity-dependent phosphorylation of WAVE1 (Sung et al., 2008). After exposure to the growth factor lysophosphatidic acid, the GTPase RhoA sequesters mitochondria from microtubules to actin (Minin et al., 2006).

Mitochondrial transport and stability is mainly regulated by neuronal activity, which regulates the intra- and extra-cellular levels of second messengers. Elevated neuronal activity increases intracellular Ca²⁺ through voltage-dependent Ca²⁺ channels, which stops mitochondria through the mechanisms discussed above. In organotypic slices of

cerebellum, time-lapse experiments show that repetitive firing accumulates mitochondria in nodal and paranodal axoplasm and decreases the transport of mitochondria in the internodal axoplasm of nodes of Ranvier. The removal of extracellular Ca^{2+} or the blockage of voltage-dependent Ca^{2+} channels abolishes the recruitment of mitochondria to nodes of Ranvier (Ohno et al., 2011). Ca^{2+} influx also induces mitochondrial fragmentation through the activation of Drp1 (discussed above). Another study shows that the blockage of the Na^+/K^+ ATPases also halts mitochondria in nodes of Ranvier, even if the mechanism is not clear (Zhang et al., 2010). These data explain the increase in mitochondrial density in absence of myelin, where Na^{2+} channels are dispersed (Andrews et al., 2006; Hogan et al., 2009) and show the physiological function of mitochondria in neurons, that is, to maintain the homeostatic levels of Ca^{2+} and ATP. For instance, in the calyx of Held mitochondria buffer Ca^{2+} in the order of milliseconds and thus, influence the neurotransmitter release (Billups and Forsythe, 2002). Any alteration in Ca^{2+} homeostasis disrupts neuronal functions: in *Drosophila* Miro mutants mitochondria are spared from the neuromuscular junction and neurotransmitter is not longer released after prolonged stimulation (Guo et al., 2005). To the same extent, the blockage of mitochondrial stability by syntabulin depletion accelerates synaptic depression. Addition of ATP rescues this defect (Ma et al., 2009). Mitochondrial movement or stability can be regulated also by neurotransmitters or growth factors, including NGF (Chada and Hollenbeck, 2004), serotonin (Chen et al., 2007), dopamine (Chen et al., 2008) and nitric oxide (Rintoul et al., 2006; Zanelli et al., 2006). Moreover, also mitochondrial health influences the proper moving; it has been shown that high membrane potential drives mitochondria towards periphery, whereas acute depolarization induces the retrograde transport of mitochondria (Miller and Sheetz, 2004). Indeed, damaged and depolarized mitochondria are sequestered by autophagic membranes for degradation upon Parkin activation, as we will discuss below. Alteration in mitochondrial movement is reported in many neurodegenerative diseases. In Huntington's disease (HD), mutations in the huntingtin (htt) gene disrupts the association of organelles, including mitochondria, to kinesin and dynein, thus impairing their transport in cortical neurons (Chang et al., 2006). To the same extent, the AD-linked amyloid- β treatment of hippocampal neurons reduces mitochondrial transport (Rui et al., 2006) and accumulates mitochondria in AD patients (Stokin et al., 2005). Finally, two genes mutated in familiar amyotrophic lateral sclerosis (ALS), alsin and TDP-43, cause impairment in the mitochondrial trafficking in vitro (Millecamps et al., 2005; Shan et al., 2010; Wang et al., 2013).

3.5. Macro-autophagy

Autophagy is a degradative process that targets portions of cytosol or organelles to the lysosomal compartment for the breakdown and recycling of nutrients. It is composed by four stages (Fig. 3): initiation, elongation, maturation, and fusion. All these stages are controlled by more than 30 autophagy-related genes (ATGs), which have been characterized in yeast and then in mammals (Levine and Klionsky, 2004).

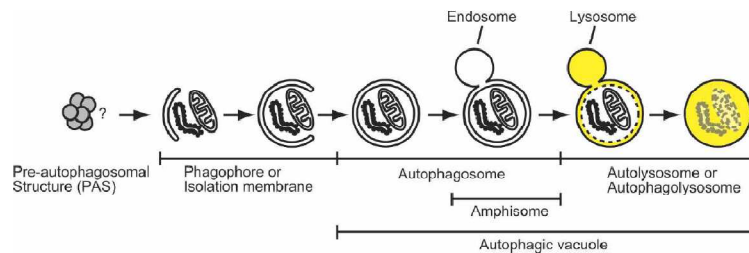


Fig.3. Schematic overview of macroautophagy. A portion of the cytoplasm containing macromolecules and organelles is engulfed by a double-membrane vesicle, the autophagosome. It subsequently fuses with the endosome and the lysosome, where the internal material is degraded. From (Mizushima, 2007).

3.5.1 Initiation

Autophagy is induced by the mammalian target of rapamycin (mTOR)-dependent or independent pathways (Fig. 4). mTOR is a nutrient sensor: in nutrient-rich conditions, mTOR is sequestered by the ULK1:ATG13:ATG101:FIP200 complex and phosphorylates ULK1 and ATG13 to inhibit autophagy (Mizushima, 2010). Deficits in autophagy induction, for example inhibition of Ulk1, suppress neurite outgrowth (Tomoda et al., 1999). On nutrient starvation, mTOR is inhibited (see below) leading to the activation of the ULK1 complex, which in turn activates the class III phosphoinositide 3-kinase (PI3K CIII) complex containing Beclin-1 and AMBRA1. This triggers the translocation of the complex to a preautophagosomal structure to initiate the autophagosome formation (Suzuki et al., 2007), even if the origin of the membrane is not univocally identified (Hayashi-Nishino et al., 2009; Yla-Anttila et al., 2009; Hailey et al., 2010; Ravikumar et al., 2010; van, V et al., 2010). This step is crucial during the development of the nervous system, since AMBRA1 deficiency prevents the closure of the neural tube (Fimia et al., 2007) and neurogenesis in cultured cells from the olfactory bulb (Vazquez et al., 2012). mTOR is regulated by different stimuli which are fully reviewed by Yang and Klionsky (2009). Briefly, the mTOR regulation is focused on the TSC-Rheb axis: in resting

conditions, the GTP-ase activating complex TSC1:TSC2 is inactive and stabilize Rheb-GTP, which activates mTOR, therefore inhibiting autophagy. The TSC complex is regulated by the PI3K-AKT pathway, which is sensitive to growth factors (insulin/IGF) and associated with autophagy during the genesis of the axon (Shi et al., 2003; Jiang et al., 2005). TSC is localized in developing axons and their overexpression causes a poor axon growth (Choi et al., 2008). Mutations of TSC1 or TSC2 cause tuberous sclerosis which specific hallmarks are non-malignant tumors in different organs. TSC model mice show cognitive impairment (Goorden et al., 2007), and impairment of hippocampal mGluR-long-term depression (LTD) (Bateup et al., 2011) and dendritic spine plasticity (Tavazoie et al., 2005). The TSC complex is activated also in response to AMPK phosphorylation; moreover AMPK activates ULK1 independently of mTOR (Lee et al., 2010b; Kim et al., 2011). AMPK is a sensor of energy deficiency and other stresses (Mihaylova and Shaw, 2011): it is activated by lowering the AMP:ATP ratio and increasing Ca^{2+} and ROS concentration.

Autophagy is regulated by other mTOR-independent pathways. In nutrient-rich conditions, the antiapoptotic protein Bcl-2 sequesters and inhibits Beclin-1; during starvation, the activation of Jun-N-terminal kinase 1 (JNK1) phosphorylates Bcl-1 which allows the release and activation of Beclin-1 (Wei et al., 2008). Another mTOR-independent pathway is activated upon elevated intracellular Ca^{2+} that triggers calpain-dependent cleavage of G stimulatory protein α and production of cAMP. cAMP, in turns, stimulates IP3 production via Epac-Rap2B-PLC cascade which induces the release of Ca^{2+} from intracellular stores (Cardenas et al., 2010).

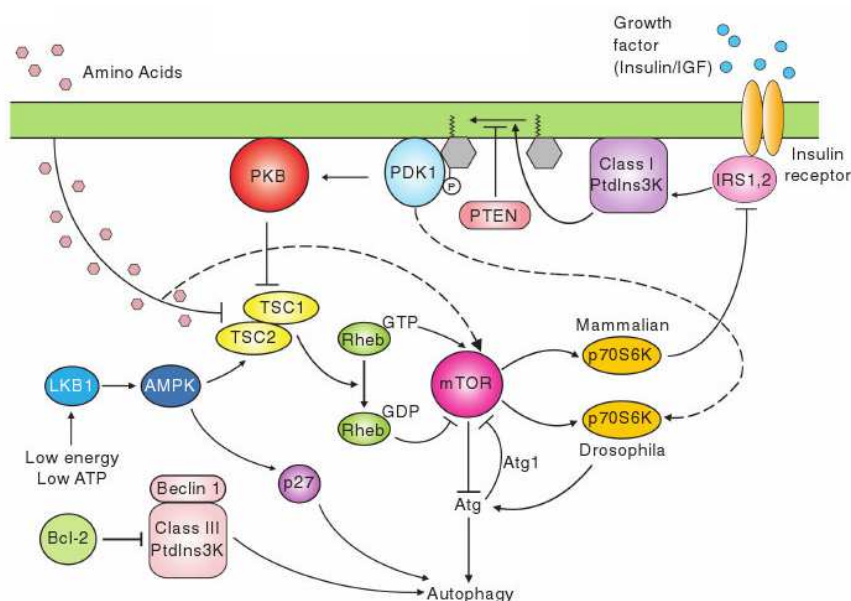


Fig. 4. Signalling pathways upstream mTORC1 regulating autophagy. From (Yang and Klionsky, 2009).

3.5.2 Elongation and maturation

After induction, the elongation and maturation of the autophagosome are sculpted by two complexes which are formed by two ubiquitin-like conjugation systems: the first conjugates Atg12 to Atg5 and the second conjugates the LC3I protein to lipid phosphatidylethanolamine to form LC3II, which is inserted in the inner and outer autophagosome membrane (Yang and Klionsky, 2009). Many Atg proteins are required for the two reactions, but they both require Atg7. Depletion of Atg7 in Purkinje cells causes accumulation of autophagosomes in the axon, leading to its degeneration (Komatsu et al., 2007). Moreover the same results are achieved by conditionally excising the Atg5 gene (Nishiyama et al., 2007), pointing out the essential role of autophagy in the maintenance of homeostasis in neurons.

3.5.3 Fusion.

When the autophagosome is formed, in neurons it is delivered to lysosomes along microtubules from distal neurites towards the soma (Maday et.al, 2012). The retrograde transport requires the dynein-dynactin complex; mutations of the dynein complex increase the autophagosome number in motor neurons of models of sporadic amyotrophic lateral sclerosis (Laird et al., 2008). Even if the fusion mechanism needs still to be elucidated, several proteins have been showed to be required, such as LAMP2, SNAREs, the UVRAG complex, Rab, ESCRT, HOPS, and LC3 (Eskelinen, 2005;Lee et al., 2007;Furuta et al., 2010). After the fusion, LC3II in the inner membrane is degraded whereas LC3II in the outer membrane is recycled by Atg4 (Kirisako et al., 2000). The material sequestered by the autophagosome is degraded by lysosomal enzymes, which are active at acid pH. Lysosomes are equipped with ionic channels and pumps to maintain the pH of 5.5 constant; mutations in any of this protein – presenilin in Alzheimer's disease (Lee et al., 2010a), TRP-ML1 in mucopolipidosis type IV (Soyombo et al., 2006), and vATPase in osteopetrosis with neurodegeneration (Wartosch and Stauber, 2010) – interfere with lysosomal acidification and therefore with autophagosome clearance. Lysosomal storage disorders (LSDs) are severe neurodegenerative disorders caused by mutations of lysosomal enzymes, which in general lead to protein aggregates unable to be digested. The most common LSD is Gaucher disease type II; mutations of the glucocerebrosidase gene (GBA) cause the accumulation of substrate within lysosomes and loss of

dopaminergic neurons in the substantia nigra, resembling clinical symptoms of Parkinson disease (Osellame and Duchen, 2013). Protein aggregates in lysosomes are found in other diseases, such as mutations of chatepsin D in Batten's disease causing the accumulation of lipofuscin (Siintola et al., 2006).

3.6. Mitophagy

3.6.1 Mitochondrial signaling for mitophagy

Mitochondria are at the center of most metabolic pathways and therefore during evolution cells developed special quality control apparatuses in order to cope with mitochondrial dysfunction. The ubiquitin-proteasome system (UPS) recognizes misfolded or damaged proteins covalently modified by a poly-ubiquitin addition, and transport them to the proteasome for degradation (Korolchuk et al., 2010). The second and more complex system is mitophagy, a selective form of autophagy of mitochondria, which appears to be finely regulated by mitochondrial dynamics. In healthy conditions, a fission event usually follows a fusion. The daughter mitochondria show different potentials; mitochondria with normal potential will undergo other fusion/fission dynamics, whereas depolarized mitochondria are degraded by mitophagy (Twig et al., 2008). Interestingly, fragmented and depolarized mitochondria are stationary (Twig et al., 2010) and degrade the pro-fusion machinery (Head et al., 2009;Poole et al., 2010;Tanaka et al., 2010b) to decrease the probability of fusion; indeed, inhibition of fragmentation reduces mitochondrial removal (Cheung and Ip, 2009). Thus, depolarization is a key signal to induce mitophagy since it is conserved also in lower eukaryotes (Priault et al., 2005). Depletion of mitochondria and accumulation of autophagosomes have been reported in brains of Alzheimer's disease (AD) patients (Nixon et al., 2005). Mitochondrial dynamics appear to be dysregulated also in AD, even if it is a complex pathology that still needs to be investigated. Amyloid β precursor protein (A β PP), mutated in familial AD, presents a mitochondrial targeting signal at the N-terminal which induces the accumulation of A β PP in the mitochondrial compartment in cortical neurons of a mouse model of AD (Devi et al., 2006), causing different mitochondrial dysfunctions when A β PP mutants are expressed. Mitochondrial

depolarization has been also reported in other protein-aggregate disorders, such as Huntington (Bossy-Wetzel et al., 2008) and Gaucher diseases (Osellame and Duchen, 2013). Mitochondria exhibit enhanced fragmentation through regulation of fusion and fission proteins (Wang et al., 2008) and complexes I, III and IV of the respiratory chain are inhibited (Pereira et al., 1998), therefore the mitochondrial potential is greatly impaired. Similarly, mutations of the mitochondrial fission/fusion apparatus induce depolarization and neurodegeneration; for example, Opa1 pathogenic mutants – associated to dominant optic atrophy – or Opa1 downregulation causes mitochondrial dysfunction in mouse embryonic fibroblasts and in cortical neurons (Frezza et al., 2006; Bertholet et al., 2013). The same phenotype is achieved by downregulation of Mfn2 – associated to Charcot Marie-Tooth 2A – in muscle and HeLa cells (Pich et al., 2005). However, mitochondrial depolarization could be the direct consequence of oxidative phosphorylation (OXPHOS) impairment: for example, complex I mutations cause mitochondrial depolarization in cybrids derived from Leber's hereditary optic neuropathy (Giordano et al., 2011). Interestingly, also PD patients show a reduced complex I activity and the associated mitochondrial depolarization. Complex I inhibitors – MPTP and rotenone – induce parkinsonism in healthy animals and degeneration of dopaminergic neurons of the substantia nigra pars compacta (Dauer and Przedborski, 2003). Pink1 and Parkin are genes mutated in certain forms of autosomal recessive PD (Kitada et al., 1998; Valente et al., 2004) and have been found to be key regulators of depolarization-induced mitophagy.

3.6.2 PINK1/Parkin regulation of mitophagy

Pink1 is a serine/threonine kinase and Parkin is a E3 ubiquitin ligase. Genetic studies in *Drosophila* and vertebrates demonstrate that the two genes participate in the same pathway to control the mitochondrial integrity, where Pink1 acts upstream of Parkin (Greene et al., 2003; Park et al., 2006; Matsui et al., 2013). Pink1 contains a mitochondrial targeting sequence (Valente et al., 2004), which allows its import in healthy mitochondria probably by the TIM/TOM complex. After the import in the inner membrane, Pink1 is cleaved by the mitochondrial proteases MPP (Greene et al., 2012) and PARL (Deas et al., 2011; Meissner et al., 2011), and subsequently degraded. In damaged mitochondria, the loss of membrane potential is the trigger event activating mitophagy: unprocessed Pink1 accumulates on the mitochondrial outer membrane where it associates with the TOM complex (Lazarou et al., 2012). Even if the mechanism is still to be clarified, uncleaved

Pink1 stabilizes cytosolic Parkin to mitochondria (Narendra et al., 2010), which in turn promotes autophagy (Narendra et al., 2008).

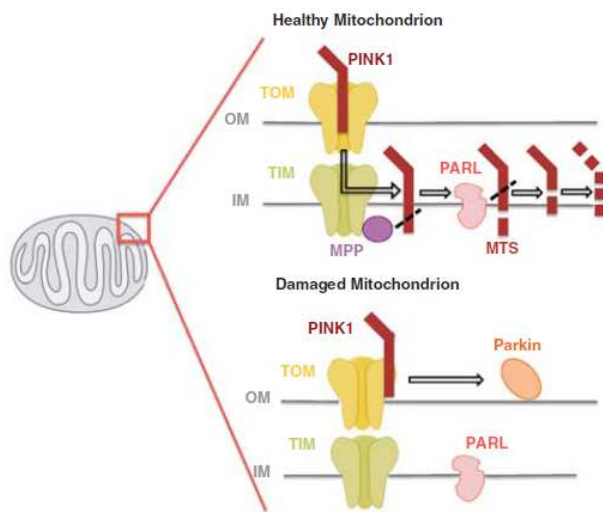


Fig. 5. The Pink1/Parkin pathway of mitophagy. In healthy mitochondria, Pink1 is imported in the inner mitochondrial membrane (IM), where is degraded by MPP and PARL. In damaged mitochondria, the loss of membrane potential stabilizes Pink1 on the outer mitochondrial membrane (OM), which induces the accumulation of Parkin on mitochondria. From (Ashrafi and Schwarz, 2013).

Stabilized Parkin enhances the E3 ubiquitin ligase activity (Matsuda et al., 2010) and forms two different polyubiquitin chains on mitochondrial proteins: ubiquitin linked to lysine 48 is associated with proteasome degradation, and polyubiquitination at lysine 63 is associated with autophagy (Tan et al., 2008). Several mitochondrial proteins have been shown to be target of Parkin before mitophagy, including Miro, Mitofusins, Fis1, Tom 70 and VDAC (Gegg et al., 2010; Geisler et al., 2010; Ziviani et al., 2010a; Chan et al., 2011). A recent screening of Parkin-mediated ubiquitylome identified dozens of proteins involved in potential Parkin regulation in mitochondrial fusion/fission, small molecule transport, apoptosis, iron-sulfur shuttling, protein translocation and proteasome assembly or activity (Sarraf et al., 2013). However, detailed downstream mechanisms activating mitophagy remain still unclear and controversial. One possible candidate is p62, which has been shown to accumulate on depolarized mitochondria (Okatsu et al., 2010), but its role in the Pink1/Parkin pathway is not clear (Geisler et al., 2010; Narendra et al., 2010). However, two independent studies suggest that Pink1 (Michiorri et al., 2010) and Parkin (Van et al., 2011) interact with Beclin1 and Ambra1, respectively, to induce autophagy. As mentioned above, Beclin1 and Ambra1 are two members of the PI3K CIII complex, which recruits membranes for the formation of the autophagosome. Parkin translocation triggers the accumulation on mitochondria of the histone deacetylase HDAC6, which binds ubiquitylated proteins, and promotes autophagy (Lee et al., 2010c). As discussed above, the Pink1/Parkin activation recruits several proteins by promoting mitochondrial fission and immobilization to facilitate autophagy. Indeed, polyubiquitinated mitofusins are removed

from mitochondria by the p97 AAA-ATPase and targeted to proteasome in neuroblastoma cell lines (Tanaka et al., 2010b) and in flies Pink1 and Parkin promotes fission (Poole et al., 2008), but it is still under debate in mammals (Ashrafi and Schwarz, 2013). Following mitochondrial depolarization, Pink1 and Parkin translocate to mitochondria and interact with Miro that anchors mitochondria to a kinesin motor complex. Phosphorylation and ubiquitination of Miro mediates its proteasomal degradation and arrest mitochondrial motility (Wang et al., 2011). Moreover, Parkin replaces degraded mitochondria with healthier ones by promoting mitochondrial biogenesis; indeed active Parkin degrades PARIS, the transcriptional repressor of PGC1 α , which induces the expression of genes promoting mitochondrial biogenesis (Shin et al., 2011). Despite the growing body of evidence pointing out the central role of the Pink1/Parkin pathway of mitophagy, studies carried on neurons are quite confusing. In these studies, Parkin translocation to mitochondria is observed when Parkin itself is overexpressed (Seibler et al., 2011; Cai et al., 2012; Joselin et al., 2012); on the contrary, in non-transfected cells Parkin translocation (Van Laar et al., 2011) or Parkin-independent mitophagy (Rakovic et al., 2013) are reported. In addition, *in vivo* experiments are far to show Parkin recruitment or mitophagy in neurons with dysfunctional mitochondria (Sterky et al., 2011; Lee et al., 2012). Dissimilar results *in vitro* could be due to different supplements in the media that may facilitate or inhibit Parkin translocation (Grenier et al., 2013). In particular, Parkin fails to translocate to mitochondria in neurons treated with supplements containing antioxidants. DJ-1, a gene linked to ROS management and mutated in recessive PD, modulates Parkin translocation and mitophagy during oxidative conditions in flies (Joselin et al., 2012), even if its role is not conserved in mammals (Haque et al., 2012). Another upstream regulator of mitophagy is p53, which sequesters Parkin in the cytosol and prevents its translocation to mitochondria (Hoshino et al., 2013).

Despite the significant body of evidence supporting the Pink1/Parkin dependence of mitophagy, new alternative regulators are emerging. Recently, cardiolipin has been proposed as mitophagy driven signal. In healthy mitochondria, cardiolipin localizes to the outer membrane, where it modulates respiratory chain complexes, autophagy and cell death (Beyer and Nuscher, 1996; Singh et al., 2010). (Chu et al., 2013) found that in response to pro-mitophagy stimuli, cardiolipin is externalized to the mitochondria surface, where it binds LC3 and induces mitophagy in cortical neurons and in a neuronal cell line. Thus, the molecular pathways regulating mitophagy are more complex than expected and further studies are needed to fully elucidate the process.

3.7. Mitochondrial dynamics and autophagy in neurodegeneration

3.7.1 Alzheimer's disease (AD)

AD is a devastating neurodegenerative disorder in which patients show an initial memory loss followed to broad cognitive dysfunction. At histological level, AD patients show extracellular deposition of amyloid plaques and accumulation of intraneuronal neurofibrillary tangles of hyperphosphorylated tau. This is coupled to synaptic loss and then neuronal death, initially in the entorhinal cortex and hippocampus, and finally more broadly in the cortex (Cummings, 2009). AD is a very complex disease, but recent evidences point out mitochondria aberrations in the disease. Indeed, mitochondrial bioenergetic deficits precede AD symptoms in a mouse model (Yao et al., 2009).

Changes in the expression of mitochondrial fission and fusion proteins has been reported by (Wang et al., 2009b), suggesting an imbalance in the fusion–fission process. Drp1 levels are increased in AD patients and it interacts with amyloid β ($A\beta$) and phosphorylated tau (Manczak and Reddy, 2012). Another study demonstrates that increased $A\beta$ fragments mitochondria and decreases mitochondrial mass in the neurites of cultured neurons. This process seems to be mediated by Drp1 activation following S-nitrosylation (Cho et al., 2009) but another study reports Drp1 phosphorylation as the activating mechanism (Bossy et al., 2010). However, the pathological meaning of these studies is unclear and other studies report normal or decreased levels of Drp1 in AD patients and animal models (Wang et al., 2009b;Bossy et al., 2010;Trushina et al., 2012). A recent study identifies the CAMKK2-AMPK pathway to mediate the early synaptotoxic effects of $A\beta$ fragments in vitro and in vivo (Mairet-Coello et al., 2013), which may also be linked to autophagy induction since $A\beta$ treatment activates the CAMKK2-AMPK pathway (Thornton et al., 2011).

Upregulated depletion of mitochondria and accumulation of autophagosome have been shown in brains of AD patients (Nixon et al., 2005). Amyloid β precursor protein ($A\beta$ PP), mutated in familial AD, presents a mitochondrial targeting signal at the N-terminal which induces the accumulation of $A\beta$ PP in the mitochondrial compartment in cortical neurons of a mouse model of AD (Devi et al., 2006), causing different mitochondrial dysfunctioning when $A\beta$ PP mutants are expressed. Indeed $A\beta$ PP overexpression fragments and clusters mitochondria in the perinuclear area, elevates reactive oxygen species, decreases

mitochondrial membrane potential, and reduces ATP production, and also reduces neuronal differentiation deficiency upon retinoic acid treatment (Wang et al., 2008).

Axonal degeneration in AD patients is characterized by regions in which mitochondria and other organelles accumulate (Stokin et al., 2005). The A β treatment of hippocampal neurons reduces mitochondrial transport (Rui et al., 2006).

In AD brains, autophagosomes accumulate in dystrophic neurites, indicating impaired autophagy (Nixon, 2007), in particular in pyramidal neurons, hippocampus, and prefrontal cortex (Nixon and Yang, 2011). mTOR signalling and the levels of lysosomal hydrolases are increased (Yang et al., 2011), possibly reflecting impaired clearance of autophagosomes/lysosomes (Boland et al., 2008). Some evidences suggest a possible role for autophagy in A β metabolism. Autophagosomes generate and contain A β (Yu et al., 2005) and oxidative stress-induced autophagy increases A β generation (Zheng et al., 2011). Moreover, induction of autophagy decreases A β levels and improves cognition (Caccamo et al., 2010). Conversely, depletion of Beclin1 increases A β load (Pickford et al., 2008), which in turn inhibits autophagy by mTOR activation (Caccamo et al., 2010). A recent study reports that autophagy influences secretion of A β to the extracellular space and thereby affecting A β plaque formation (Nilsson et al., 2013). Interestingly, Parkin clears damaged mitochondria and ubiquitinated A β in a beclin-dependent mechanism (Khandelwal et al., 2011), which can be activated also by prion protein (Nah et al., 2013).

3.7.2 Parkinson's disease (PD)

In PD, the accumulation of α -synuclein in Lewy bodies is the hallmarck preceding nigrostriatal dopaminergic neurons degeneration, which result in the characteristic deficits in movement (Braak et al., 2003). The pathogenesis of PD is still unclear but some evidences from AD patients and dopaminergic neurotoxins indicates a pivotal role for mitochondria (Schapira et al., 1990; Betarbet et al., 2000). Additional evidence for mitochondrial involvement comes from genes mutated in familial forms of PD, in particular in the autosomal recessive PD genes PINK1 and Parkin. In *Drosophila*, PINK1 and Parkin act as fission proteins and regulation of the network in absence of one or the other protein depends on the abundance of Drp1 (Poole et al., 2008; Yang et al., 2008). Parkin and PINK1 may regulate mitochondrial dynamics through their role in mitophagy and in mitochondrial motility in axons, as discussed above. Interestingly, another autosomal recessive PD protein, DJ-1, regulates mitochondrial

morphology, possibly as a consequence of increased reactive oxygen species. Parkin or PINK1 overexpression reverts the effects of DJ-1 on mitochondrial fragmentation (Irrcher et al., 2010;Wang et al., 2012a).

Mutations of PINK1, Parkin, or DJ-1 are peculiar of rare forms of PD which show degeneration mainly restricted to dopamine neurons than idiopathic PD. Familiar PD also lacks Lewy bodies, and this may restrict the relevance of this genes in idiopathic PD (Ahlskog, 2009). α -synuclein is the major component of Lewis bodies and aggregates in high molecular weight oligomers (Spillantini et al., 1998). In vitro evidences report α -synuclein localization to the IMM, where it may interact with complex I and reduce mitochondrial respiration (Devi et al., 2008;Shavali and Sens, 2008). Therefore it is not surprising that α -synuclein increases the sensitivity of dopaminergic neurons to mitochondrial toxins such as MPP⁺ and 6-hydroxydopamine (Orth et al., 2004). Indeed in α -synuclein overexpressing mice, α -synuclein is found in mitochondrial aggregates where it disrupts complex I activity and increases mitophagy (Chinta et al., 2010). Inhibition of autophagy promotes α -synuclein and LRRK2 presynaptic accumulation and causes dopaminergic axons and dendrites degeneration (Friedman et al., 2012).

Overexpression or mutation of the autosomal dominant PD proteins α -synuclein and LRRK2 can also affect mitochondrial dynamics (Kamp et al., 2010;Wang et al., 2012b). These proteins may have a role in idiopathic PD, since α -synuclein aggregates in the brains of sporadic PD patients, in Lewy bodies and Lewy neurites (Jellinger, 2012). LRRK2 is the most common genetic cause of PD (Clark et al., 2006) and regulates mitochondrial fragmentation through interaction with Drp1 (Niu et al., 2012;Wang et al., 2012b).

3.7.3 Huntington's disease (HD)

HD is a neurodegenerative disease caused by the expansion of CAG triplet repeat in the polyglutamine region of the huntingtin gene (Htt). Mutant Htt forms intracellular aggregates mainly in the striatum (Zheng and Diamond, 2012), which are coupled to mitochondrial failure (Bossy-Wetzel et al., 2004) and in particular, enhanced sensibility to complex I inhibition (Massieu et al., 2001). HD patients and HD animal models show fragmented mitochondria and decreased motility and respiration (Bossy-Wetzel et al., 2008). Indeed, increased levels of Drp1 and decreased Opa1 and Mfns are found in HD patients (Shirendeb et al., 2012), suggesting a shift toward mitochondrial fission. Interestingly, mutant Htt is found on mitochondrial surface (Panov et al., 2002), where it colocalizes with

Drp1 (Wang et al., 2009a; Song et al., 2011). Htt aggregates bind to Drp1 and increases its GTPase activity *in vitro* (Song et al., 2011). Thus, Drp1 is a good candidate in the pathogenesis of HD since the introduction of a Drp1 dominant negative reverts mitochondrial fragmentation and cell death induced by mutant Htt (Wang et al., 2009a; Song et al., 2011). Moreover, increased levels of Ca²⁺ can activate Drp1 through dephosphorylation by the calcium-dependent phosphatase calcineurin (Costa et al., 2010) and inhibition of Drp1 by FK506 reduces cell death in striatal neurons of HD models (Rosenstock et al., 2011). Recently, Htt has been proposed to regulate mitochondrial dynamics at the transcription level, possibly through PGC1 α (Cui et al., 2006).

3.7.4 Amyotrophic lateral sclerosis (ALS)

Motor neuron degeneration is the major hallmark of ALS. Familiar ALS related studies are mainly focused on mutant SOD1, which has been found in a subset of ALS patients (Duffy et al., 2011). Animal models expressing mutant SOD1 show fragmented mitochondria (Magrane et al., 2009) and the expression levels of the fusion/fission proteins are altered (Ferri et al., 2010). Mutant SOD1 disrupts mitochondrial transport and bioenergetics in motor neurons, which show tremendous synaptic alterations (Magrane et al., 2009) and defective mitochondrial transport has been suggested also in axons of ALS animal models (De Vos et al., 2007; Bilsland et al., 2010). However, this hypothesis is still debated since ablation of syntaphilin does not rescue ALS-like symptoms in G93A-SOD1 mice (Zhu and Sheng, 2011). A fraction of normal SOD1 is localized in mitochondria, where it apparently prevents oxidative damage (Fischer et al., 2011). However, mutant SOD1 is present in high levels on the OMM (Vande et al., 2008), where it associates to VDAC1 (Israelson et al., 2010). Mitochondrial mutant SOD1 affects protein import in mitochondria of spinal cord but not of other tissues (Li et al., 2010) and is necessary and sufficient to induce mitochondrial damage and cell death (Cozzolino et al., 2009; Magrane et al., 2009). TDP-43 is a DNA and RNA binding protein which is mutated in certain forms of ALS and found in aggregates in the anterior horns of spinal cord (Xu et al., 2011). Mutant TDP-43 mice show depletion of mitochondria from neuromuscular junctions, probably explained by abnormal mitochondrial trafficking due to cytoplasmic inclusions containing KIF3a (Shan et al., 2010). Another TDP-43 mice shows elevated levels of Drp1 and Fis1 and reduced Mfn1 (Xu et al., 2010).

3.7.5 Charchot-Marie-Tooth (CMT)

CMT is one of the most common inherited disorders in humans, with an estimated prevalence of 1: 2500. CMT neuropathies can be divided into two main, type 1 and type 2. In Charchot-Marie-Tooth type 1, nerve conduction velocities are considerably reduced. Charchot-Marie-Tooth type 2a (CMT2a) is due to Mfn2 mutations in the 50% of cases. In CMT2a, the nerve conduction velocities are normal but conduction amplitudes are decreased, due to the loss of nerve fibers (Zuchner et al., 2004).

CMT2a is characterized by distal muscle weakness and sensory loss, decreased reflexes and foot deformities. Other symptoms include cranial nerve involvement, scoliosis, vocal cord paresis and glaucoma. At the cellular level, CMT2 include loss of sensory and motor axons at early stages of the disease followed by the degeneration of the neurons themselves during a later stage of the disease.

The prevalence of MFN2 mutations in CMT2A are missense mutations concentrated in the GTPase and the RAS-binding domains (Kijima et al., 2005; Lawson et al., 2005; Zuchner et al., 2004). A *de novo* truncation mutation in MFN2 has been associated to CMT2 and optic atrophy (also known as hereditary motor and sensory neuropathy VI, HMSN VI) (Zuchner et al., 2006).

Loss of Mfn2 increases mitochondrial DNA mutations and reduces mitochondrial DNA copy number, resulting in impaired aerobic respiration in mice (Chen et al., 2010). An alternative study points out the improper mitochondrial trafficking in axons, since Mfn2 interacts with the Miro/Milton complex (Misko et al., 2010), which in turn may causes bioenergetic failure in the axon. Indeed, a further study shows segmental axonal degeneration without cell body death, suggesting the improper mitochondrial positioning, rather than global mitochondrial dysfunction, as the main cause of CMT2a (Misko et al., 2012).

Mutations in the ganglioside-induced differentiation-associated protein 1 (GDAP1) are associated to Charchot-Marie-Tooth type 4a (Niemann et al., 2005). GDPA1 is an outer-membrane protein which is involved in fission (Niemann et al., 2009; Estela et al., 2011).

3.8. The retina: anatomy and development

The eye is a structure which derives from different embryonic cellular populations, that is, the anterior neuroectoderm, the epidermic neuroectoderm and the neural crests. The morphogenesis of the eye begins after the closure of the neural tube, when two optic vesicles emerge from the diencephalic vesicle. The optic vesicle moves laterally and form a bistratified structure (the optic cup), which will generate the neural retina (internal layer) and the surrounding pigmented epithelium (external layer).

The retina is a pluristratified structure. In transversal sections, from the pigmented epithelium to the crystalline lens, the retina is divided in five layers (Fig. 6). The outer nuclear layer (ONL) is constituted by cell bodies of photoreceptors (rods and cones), the external plexiform layer (EPL) presents axons and dendrites of photoreceptors, bipolar cells, amacrine cells and horizontal cells, their cell bodies form the inner nuclear layer (INL). An intricate net of axons and dendrites of retinal ganglions cells and cells of INL give rise to the internal plexiform layer (IPL). Ultimately, somas of RGCs form the ganglion cell layer (GCL).

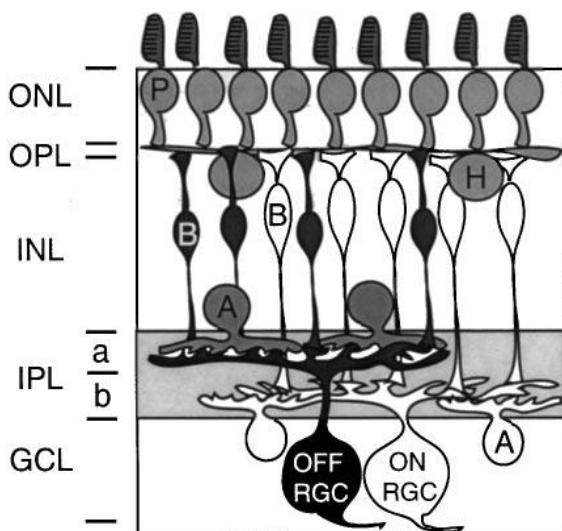


Fig. 6. Anatomy of the retina. GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; RGC, retinal ganglion cell; A, amacrine cell; B, bipolar cell; H, horizontal cell; P, photoreceptor. IPL is subdivided in sublayer a and b. From (Sernagor et al., 2001).

Cell differentiation in the retina begins from retinal progenitors in the ventricular zone, starting from the central portion of the optic cup and continuing to the periphery of the retina (Fig. 7).

RGCs are the first cells to differentiate. Following the proliferation phase (from embryonic day 11, E11 to E19 in mouse), post-mitotic cells migrate apically in the ventricular zone retaining a radial connection in the apical and basal sides of the retina. When they reach

the final position, RGCs lose radial connections and begin to establish a polarity generating axonal (start at E16) and dendritic projections (start at E17). The initial segment of axon is generated during the retraction of radial processes; it then grows towards the optic papilla and exits the lamina cribrosa in an unmyelinated state. After passing the lamina cribrosa, RGCs projections are wrapped by myelin and form the optic nerve, which convey the visual information from the retina to the lateral geniculate nucleus. It in turns projects to the primary visual cortex. The dendritic arborization of RGCs is packed in the IPL, where it establishes synapses with amacrine cells. Following RGCs, cones and horizontal cells differentiate and mature reciprocal connections, giving rise to the EPL. Vertical connections between inner and external retina are completed after differentiation of bipolar cells, which contact RGCs.

RGCs maturation depends on genetic programs and external factors, as neurotransmitters, gap junctions or surface receptors. These factors control the correct number of neuronal populations, refine synaptic connections, eliminate topographically incorrect projections and control cell density, generating the final neuronal architecture. In particular, it has been proposed that these mechanisms regulate RGCs apoptosis, since the phase of apoptosis coincides with the phase of synaptogenesis (between postnatal 2, P2 and P5 in mouse). Likewise, rats show the 50% of apoptosis during the formation of synapses (Perry et al., 1983) and eliminate 90% of RGCs in the first week after birth (Galli-Resta et al., 2008).

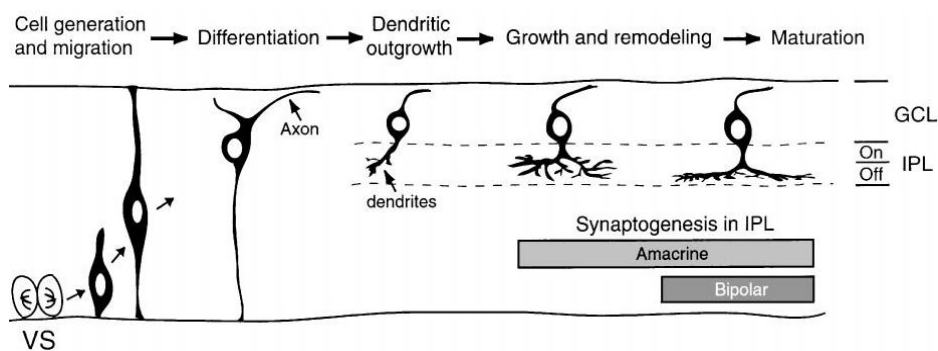


Fig. 7. RGCs development. From left to right, postmitotic RGCs migrate apically from the ventricular surface to the ganglion cell layer, where they generate axons and dendrites. Dendrites of ON or OFF RGCs stratifies in the internal plexiform layer or in the outer plexiform layer, respectively. Stratification occurs during the establishing of synapses with amacrine and bipolar cells. GCL, ganglion cell layer; IPL, inner plexiform layer; VS, ventricular surface. From (Sernagor et al., 2001).

3.9. Retinal ganglion cells

RGCs elaborate and send retinal information to the visual centers of the brain. These neurons are represented by different populations with different structure and function (Sernagor et al., 2001). The morphology of RGCs is not conserved. Their soma and their dendritic harborization have various dimensions and also the architecture of dendrites (Wassle and Boycott, 1991; Rodieck and Watanabe, 1993) and the pattern of axonal harborizations (Garraghty and Sur, 1993; Yamagata and Sanes, 1995a; Yamagata and Sanes, 1995b) are different.

At the functional level, the same light input causes different electrical responses among RGCs (Wassle and Boycott, 1991). Their activity can be transient or sustained, fast or slow, tonic or phasic. Some RGCs are good sensors of movement, whereas others show a preferential direction of a moving stimulus, and others are particularly sensitive to orientation of the stimulus but not to its direction. Moreover RGCs differs according to the sensibility of contrast and visual acuity. Some electrophysiological studies depict a strict relationship between morphology and function of these cells in vertebrates (Saito, 1983; Stanford and Sherman, 1984). Some data about structure and function of RGCs belonging to different species permitted to identify RGCs subclasses. In the Primate retina two functional classes of RGCs are described; type M (from latin magnus, which means big) and type P (from latin parvo, which means small). Both types include cells having ON-centre and OFF-centre.

RGCs type M present huge receptive fields and big dendritic harborizations and respond transiently to sustained illumination. They respond preferentially to big objects and follow greatly fast changes of the stimulus. They localize mainly in the peripheral retina. RGCs type P are the most abundant. Despite to RGCs type M, they are characterized by small receptive fields and are distributed in the central retina. They respond to specific wavelengths and they are involved in the perception of color and shape. P cells are essential for the perception of fine details in images, even if also some M cells behave this function.

The koniocellular type are bistratified RGCs representing the 10% of all RGCs. They have been identified very recently because of their relately small size (indeed, koniocellular means "cells as small as dust"). RGCs type K have very large receptive fields, which only have centers and are ON to the blue cone, but always OFF to the red and the green cone.

These neurons show moderate spatial resolution and respond to moderate contrast stimuli.

Photosensitive ganglion cells include mainly RGCs type M. These cells express the photopigment melanopsin, which allows them to directly respond to the light input. Photosensitive ganglion cells project to the suprachiasmatic nucleus for circadian rhythm regulation and send collaterals to the Edinger-Westphal nucleus to control the pupillary light reflex.

In the Cat retina, which is the best studied one, α RGCs and β RGCs have been identified. In general, every subclass of RGCs can be divided in depolarizing cells (ON RGCs) and hyperpolarizing cells (OFF RGCs). Every subtype shows typical features. For instance, the pattern and dimension of their dendrites are similar in any position inside the retina and their dendritic fields overlap, shaping a kind of mosaic covering the entire retinal surface (Wassle et al., 1983; Cook and Chalupa, 2000). Moreover, they receive the same complex of synaptic inputs and they project to the same regions located in the same cerebral target. However, RGCs subclasses differ and are not conserved among species, even if they share the anatomical organization. Indeed, the IPL, in which RGCs give rise to intraretinal connections, is divided in two sublayers which differs in anatomy and function. Independently of the subclass, the dendrites of ON RGCs stratify in the inner IPL (sublayer B), whereas dendrites of OFF RGCs stratify in the external IPL (sublayer A) (Famiglietti, Jr. and Kolb, 1976; Nelson et al., 1978). Cells having the dendritic arborization in both sublayers show ON and OFF responses.

3.10. Autosomal dominant optic atrophy

Autosomal dominant optic atrophy (ADOA) is the most common form of inherited optic neuropathy, with a frequency of 1:50.000 (Alexander et al., 2000a; Delettre et al., 2000a). ADOA clinically is characterized by the slow, progressive and bilateral decrease in visual acuity, by tritanopia (dyschromatopsia characterized by confusion in the blue-yellow hues), by sensitivity loss in the central visual fields, and by pallor of the optic nerve (Ferre et al., 2005; Votruba et al., 1997). Classic ADOA usually begins in early childhood, with a large variability in the severity of clinical expression, which may range from non-penetrant

unaffected cases up to very severe, early onset cases, even within the same family carrying the same molecular defect (Carelli et al., 2004;Ferre et al., 2005).

Histopathological analysis in post-mortem ADOA patients reveal the selective loss of retinal ganglion cells (RGCs) with a prevalence in the central retina and optic atrophy, characterized by loss of myelin and nerve tissue (Johnston et al., 1979;Kjer et al., 1983). Linkage studies identifies three genes associated to ADOA (Alexander et al., 2000a;Delettre et al., 2000a); Opa1, Opa3 and Opa7 and 75% of ADOA patients show mutations in Opa1 (Lenaers et al., 2012). The intriguing aspect of the disease is that RGCs are particularly affected, even if Opa1 is ubiquitously expressed throughout the body: in the heart, skeletal muscle, liver, testis, brain and retina (Alexander et al., 2000a). Moreover, OPA1 is not more abundant in RGCs than in other retinal cells (Kamei et al., 2005).

The majority of the mutations associated with ADOA are mainly concentrated in the GTPase and in the coiled coil domain of Opa1 (Ferre et al., 2005) and the 50% of mutations are predicted to lead to a truncated protein, suggesting that haploinsufficiency is the cause of the disease (Ferre et al., 2005;Pesch et al., 2001;Marchbank et al., 2002). However, nearly 40% of mutations occurs in the GTPase domain and may cause a dominant negative effect, impairing the mechanoenzymatic activity of the protein complexes. Three ADOA mouse models have been generated. The mouse model of Alavi and co-workers (2007) presents a STOP mutation at Gln 285, which causes protein truncation at the beginning of the GTPase domain. In the second model, an in-frame splice site mutation (329-355del) deletes 27 amino acids in the GTPase domain (Davies et al., 2007). Finally, a frame shift mutation (delTTAG) generates a protein lacking 58 amino acids (Sarzi et al., 2012). The mutations result from 30% to 50% reduction of OPA1 levels in heterozygous. Homozygous mutants are embryonically lethal at E8.5, E13.5 and E10.5, demonstrating the importance of Opa1 for proper development. The first two models of Opa1 in the heterozygous status display minimal retinal defects and normal myelination until 24 months and start to decrease visual function and visual evoked potentials (VEP) amplitude at 20 and 12 months, respectively (Alavi et al., 2007;Davies et al., 2007;Barnard et al., 2011). Morphologically, the only difference is reported in dendritic atrophy at 10 months (Williams et al., 2012). The Opa1^{delTTAG} mutant decrease VEP amplitude at 9 months and shows a significant axonal degeneration and demyelination at 16 months, starting from 5 months. Interestingly, electron microscopy reveals increased autophagic vesicles in optic and sciatic nerves (Sarzi et al., 2012).

However, the two Opa1 mice do not follow the early onset of ADOA and, therefore, do not represent a valid *in vivo* model of the pathology. The detailed mechanisms of the pathology remain still elusive *in vivo*, however the *in vitro* approach has been severely restrained by the lack of techniques to isolate RGCs from mouse, which is one of the most genetically engineered organisms. Recently, different methods have been reported (Hong et al., 2012; Winzeler and Wang, 2013). However, the first studies of Opa1 dysfunction have been performed on primary neurons or cell lines. RGCs display a unique distribution of mitochondria, which are particularly enriched in the unmyelinated part of the axon, which has high energy requirements to conduct action potentials since Na^{2+} channels are dispersed. There, mitochondria accumulate in varicosities of single axons, where also the high energy demand gap junctions have been described. Gap junctions are responsible for the centripetal extension of spreading neuron death, allowing the passage of Ca^{2+} and ROS (Rawanduzy et al., 1997). Conversely, the myelinated axons beyond the lamina cribrosa have lesser energy demand because the presence of saltatory conduction of Ranvier Nodes; thus requiring a smaller number of mitochondria in this region (Carelli et al., 2002). Indeed, the retina is one of the highest oxygen-consuming tissues of the body (Yu and Cringle, 2001). The RGCs layer has a richer body supply than the IPL, presenting a dense capillary network with additional radial peripapillary capillaries and astrocytic processes which help to maintain the brain blood barrier. Neuroglobin is a protein belonging to the family of globins, which contain a heme prosthetic group that allows the binding of oxygen, nitric oxide and carbon monoxide. It is speculated neuroglobin may facilitate oxygen diffusion from capillaries to mitochondria, where it matches especially to axonal mitochondria reaching a concentration up to 100 fold higher than in the brain. Neuroglobin knockdown reduces activity of respiratory complexes I and III and cause RGCs degeneration (Lechauve et al., 2012; Lechauve et al., 2013). Mutations in complex I lead to Leber's hereditary optic neuropathy (LHON), characterized by asymmetric rapid loss of central vision in young males due to selective RGCs degeneration and optic atrophy. Despite the different clinical evolution, the clinical endpoint of ADOA and LHON is identical and the few histological studies showed the same massive RGCs death respect to an intact retina. LHON electron microscopy studies show an abnormal distribution of mitochondrial number along the axon profile (Carelli et al., 2004), reflecting changing in energy requirements or impaired axonal transport of mitochondria. Therefore, RGCs may be more vulnerable to OPA1 inactivation because they could be particular susceptible to mitochondrial membrane disorders inducing

mitochondrial dysfunction or improper localization. Indeed, reports describe altered mitochondrial ATP synthesis and respiration in OPA1-inactivated cells (Amati-Bonneau et al., 2005;Chen et al., 2005). Moreover, recent studies show the effect of mitochondrial morphology regulation by OPA1 on mitochondrial distribution in primary cortical neurons and their contribution to dendrite formation and synaptic plasticity, which may affect the overall neuronal maturation (Bertholet et al., 2013). Indeed, Opa1 downregulation fragments and aggregate the mitochondrial network in primary rat RGCs (Kamei et al., 2005), altering essential mitochondrial functions in RGC neurons, as Ca^{2+} homeostasis (Kushnareva et al., 2013). Additionally, the defects in ADOA can be ascribed to the loss of the crucial control exerted by OPA1 on the structural organization of the *cristae* and apoptosis (Olichon et al., 2003b;Griparic et al., 2004b;Lee et al., 2004b;Arnoult et al., 2005;Frezza et al., 2006a). Indeed, Opa1 upregulation restores mitochondrial morphology and protects neurons from excitotoxic cell death (Jahani-Asl et al., 2011).

3.11. Calcineurin

Calcineurin (Cn) is a Ser/Thr phosphatase. It consists on a heterodimer of a catalytic A subunit (CnA) and a regulatory B subunit (CnB). Cn activation requires CnB and calmodulin: CnB is tightly associated with CnA thanks to two high affinity Ca^{2+} -binding sites that stabilize the heterodimer; other two low affinity Ca^{2+} -binding sites serve as Ca^{2+} sensors, since their occupancy results in the calmodulin binding to Cn and allows the full activation of the enzyme (Yang and Klee, 2002). Cn is inactive at resting Ca^{2+} concentrations (100 nM or less), but the activity is considerably increased in the range of a few hundred nM to a few μ M (Stemmer and Klee, 1994). However, prolonged stimulation inactivates Cn through a time-dependent accumulation of superoxide ions (Bito et al., 1996). Cn is distributed throughout the body, but it is particularly enriched within post-synaptic densities and cell soma of neurons in the central nervous system (Kincaid et al., 1986) with some exceptions; in fact, in the developing retina RGCs are the only neurons expressing Cn, which then becomes consistently expressed by mature amacrine cells (Nakazawa et al., 2001). Cn has a restively narrow substrate specificity and it can also indirectly regulate other proteins in neurons through regulation of protein phosphatase 1 (PP1) (Winder and Sweatt, 2001). In neurons, Cn regulates stabilization of microtubules,

neurotransmitter synthesis and release, vesicle recycling, activity of neurotransmitter receptors and gene expression (Groth et al., 2003). Cn has a central role in mitochondrial dynamics, since it is required for the Drp-1 dependent fission of mitochondria (Cereghetti et al., 2008b) and improves mitochondrial movement in glutamate-treated hippocampal neurons (Han et al., 2008b).

3.12. AMPK

AMP-activated protein kinase (AMPK) is a Ser/Thr kinase with a key role in maintaining energy metabolism at cellular and body levels. Mammalian AMPK is a complex composed by catalytic α subunit ($\alpha 1$ and $\alpha 2$ isoforms), regulatory β ($\beta 1$ and $\beta 2$), and γ ($\gamma 1$, $\gamma 2$ and $\gamma 3$) subunits. The AMPK subunits display tissue-specific expression patterns. In the nervous system, the $\alpha 2$ subunit is predominant in adult brain and spinal cord with the highest expression in neurons of cortex and hippocampus and in Purkinje cells in the cerebellum (Turnley et al., 1999). The $\gamma 1$ subunits share the same expression pattern of $\alpha 2$ subunit (Turnley et al., 1999). The $\alpha 1$ subunits is mainly expressed in embryos (Culmsee et al., 2001). $\beta 1$ and $\beta 2$ subunits are expressed in adult neurons of the whole nervous system (Turnley et al., 1999). AMPK subunits are expressed in activated astrocytes. AMPK is a sensor of energy deficiency and other stresses (Mihaylova and Shaw, 2011): it is activated by a low AMP:ATP ratio and high Ca^{2+} and ROS concentrations. There are three kinases which activate AMPK by phosphorylating Thr172 in the regulatory T-loop of the α subunit. Ca^{2+} /calmodulin-dependent protein kinase 2 (CaMKK2) (Woods et al., 2005), liver kinase B1 (LKB1) (Jansen et al., 2009), and transforming growth factor- β -activated protein kinase 1 (TAK1) (Xie et al., 2006). Conversely, protein phosphatases PP2C α and PP2A are crucial inhibitors of AMPK (Sanders et al., 2007; Wu et al., 2007). AMPK stimulates energy production via glucose and lipid metabolism; it increases fatty acid oxidation and mitochondrial biogenesis through transcriptional regulation (Hardie, 2008). Indeed, AMPK controls the cellular stress defence through a downstream network of signalling pathway, including cAMP-responsive element binding protein (CREB), forkhead box O (FOXO), peroxisome proliferator-activated receptor 1 α co-activator (PGC1 α) and silent information regulator I (SIRT1) and mammalian target of rapamycin (mTOR) (Canto and Auwerx, 2010).

AMPK is one of the major activators of autophagy by a double-pronged mechanism in which it activates unc-51-like kinase (ULK1) and inhibits mTOR through phosphorylation of the mTOR regulators TSC2 and Raptor (Lee et al., 2010b; Kim et al., 2011). AMPK controls microtubule-based transport, since it phosphorylates and inactivates Tau (Thornton et al., 2011; Mairet-Coello et al., 2013) and kinesin light chain (Amato et al., 2011), inhibiting protein and organelle trafficking in neuronal compartments; therefore reducing axonal growth and dendritic spine maintenance.

4. Results

A localized autophagic filter prevents entry of mitochondria carrying pathogenic Opa1 mutations in retinal ganglion cell axons

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Character count: 19.514

Summary

Mutations in proteins that control mitochondrial shape result in neurodegenerative diseases like Autosomal Dominant Optic Atrophy (ADOA), associate to mutated Optic Atrophy 1 (Opa1) and caused by retinal ganglion cell (RGC) loss. Intense research on Opa1 elucidated its multiple functions in mitochondrial fusion, apoptosis and metabolism, but the pathomechanisms of ADOA remain unknown. Here we show that an autophagic filter reduces axonal mitochondria in RGCs expressing pathogenic Opa1. Mutated Opa1 triggers a loop of mitochondrial dysfunction and localized autophagosome accumulation at the axonal hillock. Pharmacological or genetic inhibition of autophagy restores axonal mitochondrial entry and rescues RGCs from excess apoptosis caused by mutated Opa1. Thus localized autophagy contributes to define axonal mitochondria and pathogenesis of ADOA.

Introduction

Mitochondria possess several mitochondria shaping proteins responsible for their very dynamic nature. Mitochondrial fusion is regulated by two Mitofusins (MFN1 and MFN2) and Optic Atrophy 1 (OPA1). Mfns orchestrate outer mitochondrial membrane fusion (Santel and Fuller, 2001a;Legros et al., 2002a;Chen et al., 2003a;Santel et al., 2003a), while OPA1 cooperates with MFN1 to fuse the inner mitochondrial membrane (Cipolat et al., 2004b). OPA1 has an additional role in regulating *cristae* shape, preventing cytochrome c-dependent apoptosis and maintaining mitochondrial respiration (Cogliati et al., 2013;Zanna et al., 2008;Cipolat et al., 2006b;Frezza et al., 2006a). Moreover, MFN2 has an additional role in ER-mitochondria tethering (de Brito and Scorrano, 2008b). Dynamin related protein 1 (DRP1) is a cytoplasmic protein that during fission following its calcineurin dependent dephosphorylation (Yoon et al., 2001;Smirnova et al., 2001a;Cereghetti et al., 2008a) translocates to mitochondria where it binds to adapters such as mitochondrial fission factor (MFF), Fission 1 (Fis1) and Mitochondrial division (MiD) 49 and 51 (Loson et al., 2013).

Mitochondrial morphology and function are intimately related (Campello and Scorrano, 2010). In neurons a further complication exists in that mitochondria must be transported along microtubules to sites of high energy demand such as Ranvier Nodes and synapses, where they buffer Ca^{2+} and produce ATP to sustain neuronal activity (Sheng and Cai, 2012;Itoh et al., 2013), and by the high Ca^{2+} fluxes of these cells. Indeed, Ca^{2+} can regulate mitochondrial trafficking through the Miro/Trak complex (MacAskill et al., 2009;Wang and Schwarz, 2009b), and Ca^{2+} plus ATP dock mitochondria to Ranvier Nodes (Zhang et al., 2010;Ohno et al., 2011). High Ca^{2+} concentrations can also be deleterious for mitochondria: they can trigger the permeability transition causing their depolarization (Scorrano et al., 1997). These stationary, depolarized mitochondria can be easily engulfed by a double-membrane vesicle, the autophagosome, and degraded by mitophagy, a selective form of mitochondrial autophagy (Twig et al., 2010) which targets depolarized mitochondria through the Pink1/Parkin pathway (Greene et al., 2003;Park et al., 2006;Narendra et al., 2008;Matsui et al., 2013;Gegg et al., 2010;Geisler et al., 2010;Ziviani et al., 2010a;Chan et al., 2011). In addition, mitochondrial depolarization promotes a compensatory loop that consumes ATP, increasing AMP levels (St-Pierre et al., 2000) activating AMPK which in turn triggers autophagy (Mihaylova and Shaw, 2011).

Opa1 is mutated in Autosomal Optic Atrophy (ADOA), the most common inherited optic neuropathy with an incidence of 1:50.000 (Alexander et al., 2000a;Delettre et al., 2000a). ADOA symptoms include tritanopia, progressive loss of central vision, and optic nerve pallor (Ferre et al., 2005;Votruba et al., 1997). Histological studies in post-mortem ADOA patients showed selective loss of central retinal ganglion cells (RGCs) and optic atrophy, with demyelination and loss of nerve tissue (Johnston et al., 1979;Kjer et al., 1983). RGCs are therefore the major target of ADOA, even if Opa1 is ubiquitously expressed in all retinal layers and other tissues (Alexander et al., 2000a;Kamei et al., 2005). The majority of the ADOA-associated mutations cluster in the GTPase and in the coiled coil domain of Opa1 (Ferre et al., 2005) and ~50% of them are predicted to lead to a truncated protein (Ferre et al., 2005;Pesch et al., 2001;Marchbank et al., 2002). Three ADOA mouse models have been developed, carrying the most frequent mutations described in patients (Alavi et al., 2007;Davies et al., 2007;Barnard et al., 2011). In the mutant mice, mitochondria appear granular, are depleted from dendrites and display disorganized *cristae* (Alavi et al., 2007;Davies et al., 2007;Williams et al., 2012), yet these mice develop visual loss only around 24 months, when an ultrastructural analysis indicated also the accumulation of autophagosomes in the optic nerve (Davies et al., 2007;White et al., 2009). Accordingly, mutated Opa1 causes mitochondrial fragmentation, aggregation and depletion from dendrites as well as *cristae* derangement when expressed in primary neurons (Kamei et al., 2005;Bertholet et al., 2013). These morphologically altered mitochondria are also dysfunctional: they are unable to buffer Ca^{2+} and to maintain the membrane potential (Dayanithi et al., 2010;Bertholet et al., 2013;Kushnareva et al., 2013).

Despite the availability of mouse models and intense studies in cell cultures, ADOA pathophysiology is not well understood. A plausible explanation resides in the peculiar anatomy of RGCs, which are enriched in mitochondria in the proximal part of the axon, where energy demand to sustain action potentials is high (Carelli et al., 2004). Yet, a molecular explanation that unifies the observations in cellular and mouse models is lacking. We therefore set out to investigate the molecular mechanisms linking mutated Opa1 to death of primary RGCs isolated from mouse (Hong et al., 2012;Winzeler and Wang, 2013). We discovered that an autophagic filter actively degrades mitochondria in the soma, impairing axonal mitochondrial entry and survival in RGCs expressing pathogenic Opa1 mutants.

Results

OPA1 mutants alter mitochondrial distribution in enriched primary RGCs

Histopathology analysis from post-mortem ADOA patients show specific RGCs loss in the context of an otherwise intact retina (Johnston et al., 1979;Kjer et al., 1983). Despite the availability of animal models (Alavi et al., 2007;Davies et al., 2007;Barnard et al., 2011), the molecular mechanisms causing ADOA are still unclear and our knowledge on OPA1 functions derives from cellular models poorly related to RGCs (Misaka et al., 2002;Olichon et al., 2003b;Griparic et al., 2004b;Cipolat et al., 2004b;Cipolat et al., 2006b;Frezza et al., 2006a;Bertholet et al., 2013). Recently, magnetic labeling and immuno-panning have been described to purify RGCs from mouse (Hong et al., 2012;Winzeler and Wang, 2013), allowing to bypass the poorly informative experiments performed in total retinal cellular populations where RGCs represent a minor population (Barres et al., 1988;Williams et al., 2012). We purified RGCs via magnetic labeling and immuno-panning (Fig.1A,S1A) and we evaluated the enrichment by immunocytochemistry of the RGCs marker Brn3a and RT-PCR of markers described for other retinal cells (Fig.1B,S1B). Magnetic labeling enriched RGCs cultures up to 30% (Fig.1C), whereas immuno-panning excluded all contaminants, with the exception of macrophages and endothelial cells which express the same marker used for RGCs selection (Barres et al., 1988) (Fig. S1B). A point Lys 301 to Ala mutation (OPA1^{K301A}) and a truncated OPA1 due to the introduction of a stop codon in position 905 (OPA1^{R905STOP}) mimic the most common ADOA OPA1 mutations (Griparic et al., 2004b). Independently from the RGC isolation technique, the pathogenic mutants OPA1^{K301A} and OPA1^{R905STOP} fragmented mitochondria, whereas OPA1 and a constitutively active OPA1^{Q297V} mutant (Yamaguchi et al., 2008) increased mitochondrial length in neurites (Fig.1D,E and S1C). Mitochondria in the soma qualitatively reflect the morphology of mitochondria in the axon, but their dense packing impeded to perform a quantitative morphometric analysis. Since pathogenic OPA1 mutants have been shown to alter mitochondrial function in other cellular models (Frezza et al., 2006a;Dayanithi et al., 2010;Bertholet et al., 2013;Kushnareva et al., 2013), we wished to verify if the same was true in RGCs. Somatic as well as neurites OPA1^{K301A} and OPA1^{R905STOP} but not OPA1 and OPA1^{Q297V} mitochondria lost their membrane potential when the reversal of the mitochondrial ATPase was inhibited with oligomycin (Fig.1F), a well-established assay for latent mitochondrial dysfunction (Irwin et al., 2003). These data confirm previous observations from other cellular models and show that the method of RGC purification does not bias the effect of Opa1 on RGC mitochondria, allowing us to use the more

efficient magnetic labeling purification. Since mitochondrial depolarization triggers their axonal retrograde transport (Miller and Sheetz, 2004), we investigated mitochondrial distribution upon expression of pathogenic Opa1 mutants. The fragmented ADOA mitochondria accumulated in proximity of the axonal hillock and were depleted from the axon, without loss of cellular processes or microtubule alteration. The few mitochondria retrieved in the axon were also fragmented. Conversely, OPA1 and OPA1^{Q297V} did not alter the normal distribution and elongated morphology of mitochondria in the soma and in the axon of RGCs (Fig.2A,B and S2). We next tested whether alterations in mitochondrial transport could explain the observed loss of axonal mitochondria. Kymographic analysis revealed no differences in anterograde or retrograde mitochondrial velocities among the studied Opa1 mutants. However, fragmented ADOA mitochondria were more stationary, whereas mitochondrial trafficking was not altered in RGCs expressing OPA1 and OPA1^{Q297V} (Fig.2C,D,E). Taken together, these data confirm the mitochondrial morphological abnormalities reported in other cellular models and indicate that Opa1 mutants alter mitochondrial axonal distribution in RGCs, similarly to what observed in dendrites of cortical neurons (Bertholet et al., 2013) and RGCs (Fig.S2A,S2B).

Mitochondria expressing pathogenic Opa1 mutants display hallmarks of mitophagy

Since mitochondria expressing the pathogenic Opa1 mutants display all the hallmarks preceding mitophagy (depolarization, fragmentation and immobilization), (Twig et al., 2008; Twig et al., 2010; Head et al., 2009; Poole et al., 2010; Tanaka et al., 2010b), we tested the hypothesis that they are excluded from axons because of increased autophagy. Measurements of autophagic vesicles by expression of a yellow fluorescent protein (YFP)-LC3 sensor did not indicate gross changes in steady state autophagosome accumulation or in autophagic flux (Fig.S4), whereas it showed that autophagosomes localized at the axonal hillock when pathogenic Opa1 mutants were expressed (Fig.3A,B). The autophagosomes accumulated proximal to the fragmented mutant Opa1 expressing mitochondria (Fig.3C,D) and were basically absent from axons and dendrites (Fig.3E). Indeed, mutant OPA1 expressing mitochondria were significantly engulfed by autophagic vesicles (Fig.S3A,S3B) and overexpressed Parkin decorated OPA1^{K301A} and OPA1^{R905STOP} mitochondria, whereas it remained cytosolic in OPA1 and OPA1^{Q297V} expressing RGCs (Fig.S3C,S3D), suggesting that ADOA mitochondria sent pro-mitophagy signals. Thus, in RGCs expressing pathogenic OPA1 mutants autophagosomes accumulate and engulf mitochondria at the axonal hillock.

AMPK and Ca²⁺/calcineurin sustain the localized RGC autophagy

We next addressed the molecular mechanisms leading to localized autophagosome accumulation. AMPK activates unc-51-like kinase (ULK1) and inhibits mammalian target of rapamycin (mTOR) (Lee et al., 2010b; Kim et al., 2011) to trigger autophagy. We reasoned that AMPK could be locally activated by mitochondrial depolarization at the axonal hillock. Because active AMPK is phosphorylated on Thr172 by the AMP-dependent kinase LKB1 (Jansen et al., 2009) as well as by the Ca²⁺-dependent kinase CaMKK2 (Woods et al., 2005), we investigated the subcellular localization of Thr172 phosphorylated AMPK. Interestingly in RGCs expressing pathogenic Opa1 phosphorylated AMPK accumulated proximal to the axonal hillock, where mitochondria and autophagosome congregated. Conversely, a low signal of phosphorylated AMPK was diffuse in the soma in OPA1 and OPA1^{Q297V} expressing RGCs (Fig.4A,B). A non-phosphorylatable Thr 172 to Ala AMPK mutant (AMPK^{T172A}) which almost completely abolishes its kinase activity (Stein et al., 2000), corrected mitochondria and autophagosomes distribution only partially (Fig.4C,D and E), which was however sufficient to allow axonal mitochondrial entry.

Since AMPK was not the sole player regulating mitochondrial and autophagosome distribution in ADOA RGCs, we decided to modulate its upstream regulators. Ca²⁺ chelation by a cell permeant chelator in OPA1^{K301A} and OPA1^{R905STOP} expressing RGCs fully corrected mitochondria and autophagosomes distribution in soma and axon (Fig.4F-I). The Ca²⁺-dependent phosphatase calcineurin (Cn) is expressed in RGCs but not in the other cells of the retina (Nakazawa et al., 2001) and it restores mitochondrial motility in cells blocked by Ca²⁺ (Han et al., 2008b), making it an attractive candidate downstream Ca²⁺. A dominant negative mutant of Cn (delCnA^{H151Q}) (Cereghetti et al., 2008a) fully corrected mitochondrial and autophagosomes clustering (Fig.4J-M); moreover, a constitutively active mutant of Cn (delCnA) (Cereghetti et al., 2008a) was able to induce axonal hillock accumulation of mitochondria and autophagosomes even when OPA1 and OPA1^{Q297V} were expressed (Fig.4J-M). Interestingly, delCnA^{H151Q} did not alter the autophagic flux in OPA1^{K301A} overexpressing RGCs (Fig.S4), suggesting that the position of autophagosomes, instead of degradation efficiency, is the main regulator of mitochondrial distribution. In conclusion, a Ca²⁺-dependent mechanism involving Cn and AMPK controls autophagosome and mitochondrial accumulation.

An autophagic filter compromises mitochondrial axonal mobility and RGCs survival

If our model of abnormal mitochondrial distribution caused by localized autophagy was correct, mitochondria shall enter into the axons if autophagy is inhibited. Inhibition of autophagosome induction with 3-Methyladenine and of autophagosome fusion with the lysosome with Bafilomycin A1 fully restored axonal entry of OPA1^{K301A} and OPA1^{R905STOP} mitochondria (Fig.5A,B), despite that mitochondria and autophagosomes remained accumulated in the axonal hillock (Fig.S5A,S5B). Moreover, blockage of autophagy by 3-Methyladenine and of autophagosomal axonal hillock localization by delCnA^{H151Q} corrected the increased susceptibility of RGCs expressing OPA1^{K301A} and OPA1^{R905STOP} to apoptosis even in presence of pathogenic concentrations of Ca²⁺ triggered by glutamate (Fig.5C). The blockage of autophagy trough drastically promoted RGCs survival, providing evidence that mitochondria distribution dictates RGCs susceptibility to cell death. Thus, inhibition of autophagy restores mitochondrial distribution and viability of RGCs carrying ADOA mutations.

Discussion

Our data demonstrate that a form of localized autophagy defines mitochondrial distribution in highly polarized cells like neurons. By using RGCs and pathogenic Opa1 mutants as a model, we discovered that mitochondrial dysfunction results in accumulation of mitochondria at the axonal hillock, where they trigger local Ca^{2+} /calcineurin/AMPK dependent autophagosome buildup and where they are marked for autophagic degradation. When autophagy is inhibited, mitochondria can escape this filter and enter into the axon.

Mitochondrial motility is a highly regulated process where motors, anchors adaptors and sensors cooperate to define organellar speed, direction and position. Our results provide evidence that autophagy contributes in determining mitochondrial subcellular distribution at least in highly polarized cells like RGCs and other neurons. Whether the same system works in other cell types and for other organelles is of great interest and remains to be explored. Interestingly, evidence of increased autophagy has been collected in animal models of ADOA where Opa1 is haploinsufficient, but its relevance in the pathogenesis of the disease was unclear. Our data connect autophagy to the essential role of axonal mitochondria for RGCs survival and lend support to the so called anatomical hypothesis of ADOA, according to which the initial axon is the most vulnerable part of RGCs (Carelli et al., 2004).

RGCs belong to a subset of neurons having long axons that are the first to degenerate in many neurodegenerative diseases, including neurons of superordinate centers of somatomotor, visceromotor, and limbic systems in Parkinson's disease (Braak et al., 2004) and motoneurons affected in Amyotrophic lateral sclerosis and Charcot-Marie-Tooth 2a (Sau et al., 2011). In all these diseases, trafficking of dysfunctional mitochondria is altered (Orth et al., 2004; De Vos et al., 2007; Sasaki and Iwata, 2007; Bilslund et al., 2010; Misko et al., 2010; Misko et al., 2012) and autophagosomes aggregate in the soma (Sasaki et al., 2005; Chinta et al., 2010). However, whether the two are functionally linked and this connection is pathophysiologically relevant has never been explored. Our data indicate that these two processes are intimately linked and open the possibility to treat ADOA and other neurological disorders where mitochondria are defective and neurons with a long axon degenerate.

It is surprising that axon mitochondrial re-distribution is sufficient to correct viability of ADOA RGCs. Our data indicate that mitochondria are collectively dysfunctional and indeed, they are massively decorated with Parkin that accumulates on depolarized

mitochondria. It is therefore likely that upon autophagy inhibition, the mitochondria entering in the axon are still diseased. It is possible that the dysfunctional mitochondria accumulating in the soma amplify apoptosis, and by allowing their axonal re-entry apoptosis is reduced. Functional mitochondria are however essential to provide ATP and buffer Ca^{2+} to sustain the electrical activity of neurons: further studies are required to understand whether inhibition of autophagy fully restores neuronal functions.

The presence of autophagy has always been considered protective in neurons. Surprisingly, in our study its inhibition mechanism improves cell survival. In principle, dysregulated and aspecific autophagy triggered by mitochondrial dysfunction could impair entry in the axon of other organelles and molecules, a condition reported in many neurological disorders (Sau et al., 2011) and an amplifying factor for cell death. However, we measured autophagy-related benefits in the short period and a major caveat is that when a quality-control apparatus is blocked, dysfunctional mitochondria may take over the functional one in the long run, further precipitating the pathological condition. Thus, for how tempting it is to extend our findings to the possibility of treating neurodegenerative diseases, our model shall be tested *in vivo* in models of chronic mitochondrial dysfunction and autophagy inhibition.

Experimental procedures

Molecular Biology

peYFP-hLC3 (YFP-LC3) was kindly provided by Dr. M. Sandri (Venetian Institute of Molecular Medicine, Padua, Italy). Mitochondrially targeted dsRED (mtRFP) was a kind gift from M. Zaccolo (Venetian Institute of Molecular Medicine, Padua, Italy). pDCRHA-CnA was from Dr. S. Schiaffino (Venetian Institute of Molecular Medicine, Padova, Italy). pEYFP-C1-Parkin was a gift from Dr. E. Ziviani (Venetian Institute of Molecular Medicine, Padua, Italy). pDCRHA-CnA^{H151Q}, pEYFP-Mito (mtYFP), mito-dsRED (mtRFP) were described (Cereghetti et al., 2008b).

pMSCV, pMSCV-OPA1, pMSCV-OPA1^{K301A} and pMSCV-OPA1^{R905STOP} were described (Cipolat et al., 2004b) and pMSCV-OPA1^{Q297} (Yamaguchi et al., 2008). pCMV-AMPK^{T172A} mutant (described by (Stein et al., 2000)) was prepared from wt (provided by Dr. Campello) by site directed point mutagenesis.

All constructs were confirmed by sequencing.

To generate pCMV-AMPK^{T172A} mutant site directed mutagenesis was performed using the following primer sequence: 5'- GAATTTTAAAGAGCAAGTTGTGGCTC -3' and 5'- GAGCCACAACCTTGCTCTTAAAAATTC -3'.

Total RNA from 300.000 RGCs was purified with Absolutely RNA Microprep Kit (Ambion) and the full-length complementary DNA was obtained by RT-PCR. Primers against the following target sequences from the following mouse genes were synthesized: Brn3c, 5'- GTCTCAGCGATGTGGAGTCA -3' and 5'- GAGCTCTGGCTTGCTGTTCT -3'; Gfap, 5'- GCTGCGTATAGACAGGAGGC -3' and 5'- CGGCGATAGTCGTTAGCTTC -3'; Iba1, 5'- CGATGATCCCAAATACAGCA and 5'- GACCAGTTGGCCTCTTGTGT -3'; Lhx1, 5'- CAGTGTCGCCAAAGAGAACA -3' and 5'- ACCAGACCTGGATAACACGC -3'; Pecam1, 5'- GCCCAATCACGTTTCAGTTT -3' and 5'- GGCTTCCACACTAGGCTCAG -3'; Rcvr, 5'- ATTCCAAGTTTTTCCCGGAC -3' and 5'- ATTCCAAGTTTTTCCCGGAC -3'; Thy1, 5'- CGCTCTCCTGCTCTCAGTCT -3' and 5'- GCTCACAAAAGTAGTCGCCC -3'; Stx1a, 5'- ATGATGCCCAGAATCACACA -3' and 5'- ATGATGCCCAGAATCACACA -3'; Vsx2, 5'- ATCCCCCTGCCAGAGTCTAT -3' and 5'- TACAGTCCCCAGAACCTTGG -3'.

Animals and cell culture

From 8 to 10 P0-P2 C57Bl/6J mice were sacrificed in compliance to local animal welfare regulations, retinas dissected and digested with papain 15U/ml (Worthington) in Earle's

Balanced Salt Solution (Sigma) containing EDTA 0.5 mM (Sigma), β -mercaptoetanol 143 mM (Sigma), L-Cys-HCl 1 mM (Sigma), DNase I 125 U/ml (Worthington) at 33°C for 30 minutes. Digestion was blocked with ovomucoid inhibitor 1.2 mg/ml (Worthington), BSA 0.8 mg/ml and DNase I 125 U/ml (Worthington). Following dissociation, RGCs were purified with two protocols.

Immuno-panning

We modified the protocols of (Winzeler and Wang, 2013; Hong et al., 2012). Briefly, dissociated cells were incubated with α -mouse-macrophage antiserum 1:100 (Fitzgerald Industries) for 20 min and then in the subtraction plate functionalized with α -rabbit IgG antibody 1:100 (Sigma) for 45 min, which sequesters endothelial cells and macrophages (Thy1⁺ cells) from the supernatant. The supernatant was collected and incubated in the selection plate functionalized with α -Thy1 antibody (Sigma), which binds RGCs. Then, RGCs were detached using trypsin 0.25% (Invitrogen).

Magnetic separation

We modified the protocol of (Hong et al., 2012). Dissociated cells were incubated with α -Thy1 antibody conjugated to microbeads 1:100 (MiltenyiBiotec) at 4°C for 15 min. After careful washing, the cells were applied onto a MACS MS Column (MiltenyiBiotec) placed in a MiniMACS Separator (MiltenyiBiotec). The column was removed from the separator, and the retained cells were eluted as a magnetic-labeled RGC fraction.

Following purification, 100.000 cells were transfected with the indicated plasmids using Neon Transfection System (Invitrogen) according to manufacturer's instructions. Then, RGCs were seeded onto 24-mm round glass coverslips, which were coated with poly-L-ornithine 0.2 mg/ml (Sigma) and laminin 0.5 mg/ml (Roche). Cells were cultured in Neurobasal A Medium supplemented with B27 (Gibco), N2 (Invitrogen), L-glutamine 1% (Invitrogen) and NGF 25 ng/ml (BD Bioscience) at 37°C in a 5% CO₂ atmosphere.

Imaging

For confocal imaging of fixed cells, 100.000 cells seeded onto 13-mm round glass coverslips transfected and stained as indicated. Cells were placed on the stage of a Nikon Eclipse TE300 inverted microscope equipped with a PerkinElmer Ultraview LCI confocal system, a piezoelectric z-axis motorized stage (Pifoc, PhysikInstrumente, Germany), and a Orca ER 12-bit CCD camera (Hamamatsu Photonics, Japan). Cells expressing mtRFP and FITC stained were excited using the 488 nm or the 543 nm line of the HeNe laser (PerkinElmer) using a 60x 1.4 NA Plan Apo objective (Nikon). 70 confocal images of

mtRFP and FITC fluorescence were acquired along the z-axis, deconvolved and 3D reconstructed using the adequate plugins of ImageJ(National Institutes of Health, Bethesda).

For confocal imaging of living cells, 100.000 cells seeded onto 24-mm round glass coverslips transfected with mtRFP, YFP-LC3 and with the indicated plasmids. After 24 hrs, cells were placed on the stage of a laser scanning microscope (TCS SP5, Leica). Using the LasAF software (Leica), RFP and YFP were excited using the 488 nm or the 543 nm line of the HeNe and Argon with a 63X, 1.4NA objective. 30 confocal images of mtRFP and YFP fluorescence were acquired along the z-axis, deconvolved and 3D reconstructed using the adequate plugins of ImageJ.

For mitochondrial transport imaging, 100.000 cells seeded onto 24-mm round glass coverslips transfected with mtRFP and with the indicated plasmids. After 24 hrs, cells were placed on a thermostated chamber at 37°C and maintained on the stage of an Olympus inverted microscope equipped with a CellR imaging system. Sequential images of the 584 nm fluorescence emission were acquired every 1 s with a 60x, 1.4 NA objective (Olympus) using the CellR software and then processed using the straighten plug-in of Image J (Kocsis et al., 1991). Mitochondrial velocity was measured as described (Wang and Schwarz, 2009a).

For evaluation of membrane potential, 100.000 RGCs were plated on 24 mm round coverslips and loaded with tetramethyl methyl ester 0.5 nM (TMRM, Sigma) dissolved in Hanks' balanced salt solution (HBSS, Invitrogen) supplemented 10 mM HEPES pH 7.4 in the presence of cyclosporine H 2 mg/ml, a P-glycoprotein inhibitor for 30 min at 37°C. Cells were then placed on the stage of an Olympus IMT-2 inverted microscope (Melville, NY) equipped with a CellR imaging system. Cells were excited using a 525/20 BP excitation filter, and emitted light was acquired using a 570/LP filter. Imaging and analysis of TMRM fluorescence over mitochondrial regions of interest was performed as described (Scorrano et al., 2003).

Immunofluorescence

Primary RGCs were seeded onto 13-mm round glass coverslips coated with poly-L-ornitine and laminin. After 24 hrs cells were treated as indicated and fixed for 10 min at room temperature with 3.7% (w/V) formaldehyde (Sigma), permeabilized for 10 min with Triton-X-100 0.1% (Sigma), blocked for 1 hour with BSA 1% (Sigma) and incubated with primary antibodies. Staining was revealed with a goat anti-rabbit or anti-mouse IgG

conjugated to fluorescein-isothiocyanate (FITC) or tetramethylrhodamine (*TRITC*). Nuclei were stained with 4',6-diamidin-2-fenilindolo (DAPI).

The following antibodies were used: Phospho-AMPK α (Thr172) (1:200, Cell Signalling), SMI-312 (1:100; Abcam), β -Tubulin III (1:500, Sigma), Brn3a (1:500, Santa Cruz Biotechnology), Tom20 (1:1000, Santa Cruz Biotechnology).

Apoptosis

Primary RGCs were seeded onto 13-mm round glass coverslips coated with poly-L-ornitine and laminin. Cells were treated as indicated and apoptosis was induced with glutamate 100 μ M (Sigma) and glycine 20 μ M (Sigma). After 24 hours cells were fixed for 10 min at room temperature with 3.7% (w/v) formaldehyde and permeabilized for 10 min with Triton-X-100 0.1% (Sigma). Apoptosis was evaluated by tunel staining with *In Situ* Cell Death Detection Kit (Roche) following manufacturer's instructions.

Treatments

Autophagy was inhibited with Bafilomycin A1 200nM (Sigma) for 30 min or 3-Methyladenine 10mM (Sigma) for 24 hours in complete medium.

Ca²⁺ was chelated with BAPTA 40 μ M (Sigma) for 30 min.

Colocalization Analysis

Colocalization between autophagosomes and mitochondria was quantified using Manders' coefficient (de Brito and Scorrano, 2008b).

Figure legends

Figure 1. Opa1 pathogenic mutants fragment and depolarize mitochondria of primary RGCs

- (A)** Schematic representation of the magnetic isolation of RGCs. Cells dissociated from P0 murine retinas are magnetically labeled with Thy1 antibody (orange circles), a RGCs surface marker. Cells labeled with Thy1 antibody are retained on the column while unlabeled cells pass through (green circles). The column is removed from the separator. The retained cells are eluted as the enriched, positively selected cell fraction.
- (B)** Representative immunofluorescence of RGCs isolated with magnetic purification, marked with Brn3a (red). The nuclear DNA is marked by DAPI (blue). Bar, 20 μm .
- (C)** Analysis of enrichment on Brn3a cells after magnetic purification. The number of Brn3a cells is normalized to the number of total cells, positive for DAPI. The data represents the average \pm SD of 3 experiments (n=150-250 cells/experiment).
- (D)** Representative 3D reconstructions of stacks of confocal images of the fluorescence of mtRFP in primary RGCs cotransfected with the indicated plasmids and fixed after 24 hours. 70 confocal images of mtRFP fluorescence were acquired along the z-axis, deconvolved and 3D reconstructed (mtRFP) using the appropriate plugins of ImageJ. Bar, 20 μm .
- (E)** Quantitative analysis of mitochondrial length in fixed RGCs cotransfected with mtRFP and the indicated plasmids. Data represent average \pm SEM of 4 independent experiments (n=30 cells/experiment).
- (F)** Quantitative analysis of TMRM fluorescence changes over somatic mitochondria of GFP-positive RGCs. Oligomycin and FCCP were added. Data represent average \pm SEM of 6 independent experiments (n=4 cells/experiment).

Figure 2. Opa1 pathogenic mutants deplete mitochondria from axons of RGCs

- (A)** Representative 3D reconstructions of stacks of confocal images of the fluorescence of mtRFP (red) and β -tubulin III (green) in primary RGCs cotransfected with the indicated plasmids. RGCs were cotransfected with mtRFP (red) and the indicated plasmids. 24 hours after transfection cells were fixed and immunostained with β -tubulin III (green). The images were acquired and processed as described in Fig.1C. Bar, 20 μm .
- (B)** Quantitative analysis of the mitochondrial content of axons in RGCs cotransfected with

mtRFP and the cited plasmids. Data represent average \pm SEM of 4 independent experiments (n=20 cells/experiment).

(C) Representative kymographs of movies of the fluorescence of mtRFP in primary RGCs cotransfected with mtRFP and the indicated plasmids. 24 hours after transfection cells were analyzed at 37°C.

(D) Analysis of mitochondria in motion in neurites. The number of moving mitochondria is normalized to the number of total mitochondria. Data represent average \pm SEM of 5 independent experiments (n=5 cells/experiment).

(E) Analysis of anterograde (black) and retrograde (red) velocity of mitochondria. Data represent average \pm SEM of 5 independent experiments (n=5 cells/experiment).

Figure 3. Mitochondria and autophagosomes clusterize in proximity of the axonal hillock of RGCs expressing pathogenic mutants of Opa1

(A) Representative z-projects of stacks of confocal images of the fluorescence of YFP-LC3 in primary RGCs cotransfected with the indicated plasmids. Bar, 20 μ m.

(B) Quantitative analysis of autophagosomes accumulation in the axonal hillock in RGCs cotransfected with YFP-LC3 and the indicated plasmids. Data represent average \pm SEM of 6 independent experiments.

(C) Representative 3D reconstructions of stacks of confocal images of the fluorescence of mtRFP (red) and YFP-LC3 (green) in the soma primary RGCs cotransfected with the indicated plasmids. The asterisk indicates the axon. Bar, 20 μ m.

(D) Quantitative analysis of the mitochondrial accumulation in the axonal hillock. Data represent average \pm SEM of 6 independent experiments.

(E) Representative 3D reconstructions of stacks of confocal images of the fluorescence of mtRFP (red) and YFP-LC3 (green) in primary RGCs cotransfected with the indicated plasmids. Bar, 20 μ m.

Figure 4. Retrograde Ca²⁺/Calcineurin-AMPK signalling localizes autophagy at the axonal hillock

(A) Representative z-projects of stacks of confocal images of the fluorescence of phospho-AMPK (red) and GFP (green) in the primary RGCs cotransfected with the indicated plasmids. Bar, 20 μ m.

(B) Quantitative analysis of the fluorescence intensity of phospho-AMPK in the soma of RGCs. Data represent average \pm SEM of 5 independent experiments.

(C) Representative 3D reconstructions of stacks of confocal images of the fluorescence of mtRFP (red) and YFP-LC3 (green) in the soma and in the cell body of primary RGCs cotransfected with the indicated plasmids. The asterisk indicates the axon. Bar, 20 μ m.

(D) Quantitative analysis of autophagosomes accumulation in the axonal hillock in RGCs cotransfected with YFP-LC3 and the indicated plasmids. Data represent average \pm SEM of 3 independent experiments.

(E) Quantitative analysis of the mitochondrial accumulation in the axonal hillock (left) and of the mitochondrial content in the axon (right) of RGCs. Data represent average \pm SEM of 3 independent experiments.

(F) Representative 3D reconstructions of stacks of confocal images of the fluorescence of mtRFP (red) and YFP-LC3 (green) in the soma of primary RGCs cotransfected with the indicated plasmids and treated with BAPTA. The asterisk indicates the axon. Bar, 20 μ m.

(G) Quantitative analysis of autophagosomes and mitochondria accumulation in the axonal hillock in RGCs cotransfected with YFP-LC3, mtRFP and the indicated plasmids. Data represent average \pm SEM of 3 independent experiments.

(H) Representative 3D reconstructions of stacks of confocal images of the fluorescence of mtRFP (red) and YFP-LC3 (green) in primary RGCs cotransfected with the indicated plasmids and treated with BAPTA. The asterisk indicates the axon. Bar, 20 μ m.

(I) Quantitative analysis of the mitochondrial content in the axon of RGCs. Data represent average \pm SEM of 3 independent experiments.

(J) Representative 3D reconstructions of stacks of confocal images of the fluorescence of mtRFP (red) and YFP-LC3 (green) in the soma of primary RGCs cotransfected with the indicated plasmids. The asterisk indicates the axon. Bar, 20 μ m.

(K) Quantitative analysis of autophagosomes and mitochondria accumulation in the axonal hillock in RGCs cotransfected with YFP-LC3, mtRFP and the indicated plasmids. Data represent average \pm SEM of 3 independent experiments.

(L) Representative 3D reconstructions of stacks of confocal images of the fluorescence of mtRFP (red) and YFP-LC3 (green) in primary RGCs cotransfected with the indicated plasmids. Bar, 20 μ m.

(M) Quantitative analysis of the mitochondrial content in the axon of RGCs. Data represent average \pm SEM of 3 independent experiments.

Figure 5. Inhibition of macroautophagy reverts mitochondria localization and rescues ADOA RGCs viability

(A) Representative 3D reconstructions of stacks of confocal images of the fluorescence of mtRFP (red) and YFP-LC3 (green) in primary RGCs cotransfected with the indicated plasmids and treated with Bafilomicyn or 3-Methyladenine. Bar, 20 μ m.

(B) Quantitative analysis of the mitochondrial content in the axon of RGCs. Data represent average \pm SEM of 4 independent experiments.

(C) Analysis of apoptotic cells transfected with the indicated plasmids and stained by TUNEL assay. Apoptosis was induced by glutamate and autophagy was inhibited with 3-Methyladenine. The number of apoptotic RGCs is normalized to the number of total transfected RGCs. Data represent average \pm SEM of 4 independent experiments.

Reference List

- Alavi MV, Bette S, Schimpf S, Schuettauf F, Schraermeyer U, Wehr HF, Ruttiger L, Beck SC, Tonagel F, Pichler BJ, Knipper M, Peters T, Laufs J, Wissinger B (2007) A splice site mutation in the murine Opa1 gene features pathology of autosomal dominant optic atrophy. *Brain* 130:1029-1042.
- Alexander C, Votruba M, Pesch UE, Thiselton DL, Mayer S, Moore A, Rodriguez M, Kellner U, Leo-Kottler B, Auburger G, Bhattacharya SS, Wissinger B (2000) OPA1, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28. *Nat Genet* 26:211-215.
- Barnard AR, Charbel IP, Perganta G, Williams PA, Davies VJ, Sekaran S, Votruba M, MacLaren RE (2011) Specific deficits in visual electrophysiology in a mouse model of dominant optic atrophy. *Exp Eye Res* 93:771-777.
- Barres BA, Silverstein BE, Corey DP, Chun LL (1988) Immunological, morphological, and electrophysiological variation among retinal ganglion cells purified by panning. *Neuron* 1:791-803.
- Bertholet AM, Millet AM, Guillermin O, Daloyau M, Davezac N, Miquel MC, Belenguer P (2013) OPA1 loss of function affects in vitro neuronal maturation. *Brain* 136:1518-1533.
- Bilsland LG, Sahai E, Kelly G, Golding M, Greensmith L, Schiavo G (2010) Deficits in axonal transport precede ALS symptoms in vivo. *Proc Natl Acad Sci U S A* 107:20523-20528.
- Braak H, Ghebremedhin E, Rub U, Braatzke H, Del TK (2004) Stages in the development of Parkinson's disease-related pathology. *Cell Tissue Res* 318:121-134.
- Campello S, Scorrano L (2010) Mitochondrial shape changes: orchestrating cell pathophysiology. *EMBO Rep* 11:678-684.
- Carelli V, Ross-Cisneros FN, Sadun AA (2004) Mitochondrial dysfunction as a cause of optic neuropathies. *Prog Retin Eye Res* 23:53-89.
- Cereghetti GM, Stangherlin A, Martins de BO, Chang CR, Blackstone C, Bernardi P, Scorrano L (2008a) Dephosphorylation by calcineurin regulates translocation of Drp1 to mitochondria. *Proc Natl Acad Sci U S A* 105:15803-15808.
- Cereghetti GM, Stangherlin A, Martins de BO, Chang CR, Blackstone C, Bernardi P, Scorrano L (2008b) Dephosphorylation by calcineurin regulates translocation of Drp1 to mitochondria. *Proc Natl Acad Sci U S A* 105:15803-15808.
- Chan NC, Salazar AM, Pham AH, Sweredoski MJ, Kolawa NJ, Graham RL, Hess S, Chan DC (2011) Broad activation of the ubiquitin-proteasome system by Parkin is critical for mitophagy. *Hum Mol Genet* 20:1726-1737.
- Chen H, Detmer SA, Ewald AJ, Griffin EE, Fraser SE, Chan DC (2003) Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. *J Cell Biol* 160:189-200.
- Chinta SJ, Mallajosyula JK, Rane A, Andersen JK (2010) Mitochondrial alpha-synuclein accumulation impairs complex I function in dopaminergic neurons and results in increased mitophagy in vivo. *Neurosci Lett* 486:235-239.
- Cipolat S, Martins de BO, Dal ZB, Scorrano L (2004) OPA1 requires mitofusin 1 to promote mitochondrial fusion. *Proc Natl Acad Sci U S A* 101:15927-15932.
- Cipolat S, Rudka T, Hartmann D, Costa V, Serneels L, Craessaerts K, Metzger K, Frezza C, Annaert W, D'Adamo L, Derks C, Dejaegere T, Pellegrini L, D'Hooge R, Scorrano L, De SB (2006) Mitochondrial rhomboid PARL regulates cytochrome c release during apoptosis via OPA1-dependent cristae remodeling. *Cell* 126:163-175.
- Cogliati S, Frezza C, Soriano ME, Varanita T, Quintana-Cabrera R, Corrado M, Cipolat S, Costa V, Casarin A, Gomes LC, Perales-Clemente E, Salviati L, Fernandez-Silva P, Enriquez JA, Scorrano L (2013) Mitochondrial cristae shape determines respiratory chain supercomplexes assembly and respiratory efficiency. *Cell* 155:160-171.
- Davies VJ, Hollins AJ, Piechota MJ, Yip W, Davies JR, White KE, Nicols PP, Boulton ME, Votruba M (2007) Opa1 deficiency in a mouse model of autosomal dominant optic atrophy impairs mitochondrial morphology, optic nerve structure and visual function. *Hum Mol Genet* 16:1307-1318.
- Dayanithi G, Chen-Kuo-Chang M, Viero C, Hamel C, Muller A, Lenaers G (2010) Characterization of Ca²⁺ signalling in postnatal mouse retinal ganglion cells: involvement of OPA1 in Ca²⁺ clearance. *Ophthalmic Genet* 31:53-65.
- de Brito OM, Scorrano L (2008) Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature* 456:605-610.
- De Vos KJ, Chapman AL, Tennant ME, Manser C, Tudor EL, Lau KF, Brownlees J, Ackerley S, Shaw PJ, McLoughlin DM, Shaw CE, Leigh PN, Miller CC, Grierson AJ (2007) Familial amyotrophic lateral sclerosis-linked SOD1 mutants perturb fast axonal transport to reduce axonal mitochondria content. *Hum Mol Genet* 16:2720-2728.

- Delettre C, Lenaers G, Griffoin JM, Gigarel N, Lorenzo C, Belenguer P, Pelloquin L, Grosgeorge J, Turc-Carel C, Perret E, Astarie-Dequeker C, Lasquelléc L, Arnaud B, Ducommun B, Kaplan J, Hamel CP (2000) Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. *Nat Genet* 26:207-210.
- Ferre M, Amati-Bonneau P, Tourmen Y, Malthiery Y, Reynier P (2005) eOPA1: an online database for OPA1 mutations. *Hum Mutat* 25:423-428.
- Frezza C, Cipolat S, Martins de BO, Micaroni M, Beznoussenko GV, Rudka T, Bartoli D, Polishuck RS, Danial NN, De SB, Scorrano L (2006) OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion. *Cell* 126:177-189.
- Gegg ME, Cooper JM, Chau KY, Rojo M, Schapira AH, Taanman JW (2010) Mitofusin 1 and mitofusin 2 are ubiquitinated in a PINK1/parkin-dependent manner upon induction of mitophagy. *Hum Mol Genet* 19:4861-4870.
- Geisler S, Holmstrom KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, Springer W (2010) PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. *Nat Cell Biol* 12:119-131.
- Greene JC, Whitworth AJ, Kuo I, Andrews LA, Feany MB, Pallanck LJ (2003) Mitochondrial pathology and apoptotic muscle degeneration in *Drosophila* parkin mutants. *Proc Natl Acad Sci U S A* 100:4078-4083.
- Griparic L, van der Wel NN, Orozco IJ, Peters PJ, van der Bliék AM (2004) Loss of the intermembrane space protein Mgm1/OPA1 induces swelling and localized constrictions along the lengths of mitochondria. *J Biol Chem* 279:18792-18798.
- Han XJ, Lu YF, Li SA, Tomizawa K, Takei K, Matsushita M, Matsui H (2008) Involvement of calcineurin in glutamate-induced mitochondrial dynamics in neurons. *Neurosci Res* 60:114-119.
- Head B, Griparic L, Amiri M, Gandre-Babbe S, van der Bliék AM (2009) Inducible proteolytic inactivation of OPA1 mediated by the OMA1 protease in mammalian cells. *J Cell Biol* 187:959-966.
- Hong S, Iizuka Y, Kim CY, Seong GJ (2012) Isolation of primary mouse retinal ganglion cells using immunopanning-magnetic separation. *Mol Vis* 18:2922-2930.
- Irwin WA, Bergamin N, Sabatelli P, Reggiani C, Megighian A, Merlini L, Braghetta P, Columbaro M, Volpin D, Bressan GM, Bernardi P, Bonaldo P (2003) Mitochondrial dysfunction and apoptosis in myopathic mice with collagen VI deficiency. *Nat Genet* 35:367-371.
- Itoh K, Nakamura K, Iijima M, Sesaki H (2013) Mitochondrial dynamics in neurodegeneration. *Trends Cell Biol* 23:64-71.
- Jansen M, Ten Klooster JP, Offerhaus GJ, Clevers H (2009) LKB1 and AMPK family signaling: the intimate link between cell polarity and energy metabolism. *Physiol Rev* 89:777-798.
- Johnston PB, Gaster RN, Smith VC, Tripathi RC (1979) A clinicopathologic study of autosomal dominant optic atrophy. *Am J Ophthalmol* 88:868-875.
- Kamei S, Chen-Kuo-Chang M, Cazevielle C, Lenaers G, Olichon A, Belenguer P, Roussignol G, Renard N, Eybalin M, Michelin A, Delettre C, Brabet P, Hamel CP (2005) Expression of the Opa1 mitochondrial protein in retinal ganglion cells: its downregulation causes aggregation of the mitochondrial network. *Invest Ophthalmol Vis Sci* 46:4288-4294.
- Kim J, Kundu M, Viollet B, Guan KL (2011) AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol* 13:132-141.
- Kjer P, Jensen OA, Klinken L (1983) Histopathology of eye, optic nerve and brain in a case of dominant optic atrophy. *Acta Ophthalmol (Copenh)* 61:300-312.
- Kocsis E, Trus BL, Steer CJ, Bisher ME, Steven AC (1991) Image averaging of flexible fibrous macromolecules: the clathrin triskelion has an elastic proximal segment. *J Struct Biol* 107:6-14.
- Kushnareva YE, Gerencser AA, Bossy B, Ju WK, White AD, Waggoner J, Ellisman MH, Perkins G, Bossy-Wetzel E (2013) Loss of OPA1 disturbs cellular calcium homeostasis and sensitizes for excitotoxicity. *Cell Death Differ* 20:353-365.
- Lee JW, Park S, Takahashi Y, Wang HG (2010) The association of AMPK with ULK1 regulates autophagy. *PLoS One* 5:e15394.
- Legros F, Lombes A, Frachon P, Rojo M (2002) Mitochondrial fusion in human cells is efficient, requires the inner membrane potential, and is mediated by mitofusins. *Mol Biol Cell* 13:4343-4354.
- Loson OC, Song Z, Chen H, Chan DC (2013) Fis1, Mff, MiD49, and MiD51 mediate Drp1 recruitment in mitochondrial fission. *Mol Biol Cell* 24:659-667.
- MacAskill AF, Brickley K, Stephenson FA, Kittler JT (2009) GTPase dependent recruitment of Grif-1 by Miro1 regulates mitochondrial trafficking in hippocampal neurons. *Mol Cell Neurosci* 40:301-312.

- Marchbank NJ, Craig JE, Leek JP, Toohey M, Churchill AJ, Markham AF, Mackey DA, Toomes C, Inglehearn CF (2002) Deletion of the OPA1 gene in a dominant optic atrophy family: evidence that haploinsufficiency is the cause of disease. *J Med Genet* 39:e47.
- Matsui H, Gavinio R, Asano T, Uemura N, Ito H, Taniguchi Y, Kobayashi Y, Maki T, Shen J, Takeda S, Uemura K, Yamakado H, Takahashi R (2013) PINK1 and Parkin complementarily protect dopaminergic neurons in vertebrates. *Hum Mol Genet* 22:2423-2434.
- Mihaylova MM, Shaw RJ (2011) The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. *Nat Cell Biol* 13:1016-1023.
- Miller KE, Sheetz MP (2004) Axonal mitochondrial transport and potential are correlated. *J Cell Sci* 117:2791-2804.
- Misaka T, Miyashita T, Kubo Y (2002) Primary structure of a dynamin-related mouse mitochondrial GTPase and its distribution in brain, subcellular localization, and effect on mitochondrial morphology. *J Biol Chem* 277:15834-15842.
- Misko A, Jiang S, Wegorzewska I, Milbrandt J, Baloh RH (2010) Mitofusin 2 is necessary for transport of axonal mitochondria and interacts with the Miro/Milton complex. *J Neurosci* 30:4232-4240.
- Misko AL, Sasaki Y, Tuck E, Milbrandt J, Baloh RH (2012) Mitofusin2 mutations disrupt axonal mitochondrial positioning and promote axon degeneration. *J Neurosci* 32:4145-4155.
- Nakazawa A, Usuda N, Matsui T, Hanai T, Matsushita S, Arai H, Sasaki H, Higuchi S (2001) Localization of calcineurin in the mature and developing retina. *J Histochem Cytochem* 49:187-195.
- Narendra D, Tanaka A, Suen DF, Youle RJ (2008) Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. *J Cell Biol* 183:795-803.
- Ohno N, Kidd GJ, Mahad D, Kiryu-Seo S, Avishai A, Komuro H, Trapp BD (2011) Myelination and axonal electrical activity modulate the distribution and motility of mitochondria at CNS nodes of Ranvier. *J Neurosci* 31:7249-7258.
- Olichon A, Baricault L, Gas N, Guillou E, Valette A, Belenguer P, Lenaers G (2003) Loss of OPA1 perturbs the mitochondrial inner membrane structure and integrity, leading to cytochrome c release and apoptosis. *J Biol Chem* 278:7743-7746.
- Orth M, Tabrizi SJ, Tomlinson C, Messmer K, Korlipara LV, Schapira AH, Cooper JM (2004) G209A mutant alpha synuclein expression specifically enhances dopamine induced oxidative damage. *Neurochem Int* 45:669-676.
- Park J, Lee SB, Lee S, Kim Y, Song S, Kim S, Bae E, Kim J, Shong M, Kim JM, Chung J (2006) Mitochondrial dysfunction in *Drosophila* PINK1 mutants is complemented by parkin. *Nature* 441:1157-1161.
- Pesch UE, Leo-Kottler B, Mayer S, Jurklies B, Kellner U, Apfelstedt-Sylla E, Zrenner E, Alexander C, Wissinger B (2001) OPA1 mutations in patients with autosomal dominant optic atrophy and evidence for semi-dominant inheritance. *Hum Mol Genet* 10:1359-1368.
- Poole AC, Thomas RE, Yu S, Vincow ES, Pallanck L (2010) The mitochondrial fusion-promoting factor mitofusin is a substrate of the PINK1/parkin pathway. *PLoS One* 5:e10054.
- Santel A, Frank S, Gaume B, Herrler M, Youle RJ, Fuller MT (2003) Mitofusin-1 protein is a generally expressed mediator of mitochondrial fusion in mammalian cells. *J Cell Sci* 116:2763-2774.
- Santel A, Fuller MT (2001) Control of mitochondrial morphology by a human mitofusin. *J Cell Sci* 114:867-874.
- Sasaki S, Iwata M (2007) Mitochondrial alterations in the spinal cord of patients with sporadic amyotrophic lateral sclerosis. *J Neuropathol Exp Neurol* 66:10-16.
- Sasaki S, Warita H, Murakami T, Shibata N, Komori T, Abe K, Kobayashi M, Iwata M (2005) Ultrastructural study of aggregates in the spinal cord of transgenic mice with a G93A mutant SOD1 gene. *Acta Neuropathol* 109:247-255.
- Sau D, Rusmini P, Crippa V, Onesto E, Bolzoni E, Ratti A, Poletti A (2011) Dysregulation of axonal transport and motorneuron diseases. *Biol Cell* 103:87-107.
- Scorrano L, Oakes SA, Opferman JT, Cheng EH, Sorcinelli MD, Pozzan T, Korsmeyer SJ (2003) BAX and BAK regulation of endoplasmic reticulum Ca²⁺: a control point for apoptosis. *Science* 300:135-139.
- Scorrano L, Petronilli V, Bernardi P (1997) On the voltage dependence of the mitochondrial permeability transition pore. A critical appraisal. *J Biol Chem* 272:12295-12299.
- Sheng ZH, Cai Q (2012) Mitochondrial transport in neurons: impact on synaptic homeostasis and neurodegeneration. *Nat Rev Neurosci* 13:77-93.

- Smirnova E, Griparic L, Shurland DL, van der Bliek AM (2001) Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells. *Mol Biol Cell* 12:2245-2256.
- St-Pierre J, Brand MD, Boutilier RG (2000) Mitochondria as ATP consumers: cellular treason in anoxia. *Proc Natl Acad Sci U S A* 97:8670-8674.
- Stein SC, Woods A, Jones NA, Davison MD, Carling D (2000) The regulation of AMP-activated protein kinase by phosphorylation. *Biochem J* 345 Pt 3:437-443.
- Tanaka A, Cleland MM, Xu S, Narendra DP, Suen DF, Karbowski M, Youle RJ (2010) Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. *J Cell Biol* 191:1367-1380.
- Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, Stiles L, Haigh SE, Katz S, Las G, Alroy J, Wu M, Py BF, Yuan J, Deeney JT, Corkey BE, Shirihai OS (2008) Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *EMBO J* 27:433-446.
- Twig G, Liu X, Liesa M, Wikstrom JD, Molina AJ, Las G, Yaniv G, Hajnoczky G, Shirihai OS (2010) Biophysical properties of mitochondrial fusion events in pancreatic beta-cells and cardiac cells unravel potential control mechanisms of its selectivity. *Am J Physiol Cell Physiol* 299:C477-C487.
- Votruba M, Moore AT, Bhattacharya SS (1997) Genetic refinement of dominant optic atrophy (OPA1) locus to within a 2 cM interval of chromosome 3q. *J Med Genet* 34:117-121.
- Wang X, Schwarz TL (2009a) Imaging axonal transport of mitochondria. *Methods Enzymol* 457:319-333.
- Wang X, Schwarz TL (2009b) The mechanism of Ca²⁺-dependent regulation of kinesin-mediated mitochondrial motility. *Cell* 136:163-174.
- White KE, Davies VJ, Hogan VE, Piechota MJ, Nichols PP, Turnbull DM, Votruba M (2009) OPA1 deficiency associated with increased autophagy in retinal ganglion cells in a murine model of dominant optic atrophy. *Invest Ophthalmol Vis Sci* 50:2567-2571.
- Williams PA, Piechota M, von RC, Taylor E, Morgan JE, Votruba M (2012) Opa1 is essential for retinal ganglion cell synaptic architecture and connectivity. *Brain* 135:493-505.
- Winzeler A, Wang JT (2013) Purification and culture of retinal ganglion cells from rodents. *Cold Spring Harb Protoc* 2013:643-652.
- Woods A, Dickerson K, Heath R, Hong SP, Momcilovic M, Johnstone SR, Carlson M, Carling D (2005) Ca²⁺/calmodulin-dependent protein kinase kinase-beta acts upstream of AMP-activated protein kinase in mammalian cells. *Cell Metab* 2:21-33.
- Yamaguchi R, Lartigue L, Perkins G, Scott RT, Dixit A, Kushnareva Y, Kuwana T, Ellisman MH, Newmeyer DD (2008) Opa1-mediated cristae opening is Bax/Bak and BH3 dependent, required for apoptosis, and independent of Bak oligomerization. *Mol Cell* 31:557-569.
- Yoon Y, Pitts KR, McNiven MA (2001) Mammalian dynamin-like protein DLP1 tubulates membranes. *Mol Biol Cell* 12:2894-2905.
- Zanna C, Ghelli A, Porcelli AM, Karbowski M, Youle RJ, Schimpf S, Wissinger B, Pinti M, Cossarizza A, Vidoni S, Valentino ML, Rugolo M, Carelli V (2008) OPA1 mutations associated with dominant optic atrophy impair oxidative phosphorylation and mitochondrial fusion. *Brain* 131:352-367.
- Zhang CL, Ho PL, Kintner DB, Sun D, Chiu SY (2010) Activity-dependent regulation of mitochondrial motility by calcium and Na/K-ATPase at nodes of Ranvier of myelinated nerves. *J Neurosci* 30:3555-3566.
- Ziviani E, Tao RN, Whitworth AJ (2010) *Drosophila* parkin requires PINK1 for mitochondrial translocation and ubiquitinates mitofusin. *Proc Natl Acad Sci U S A* 107:5018-5023.

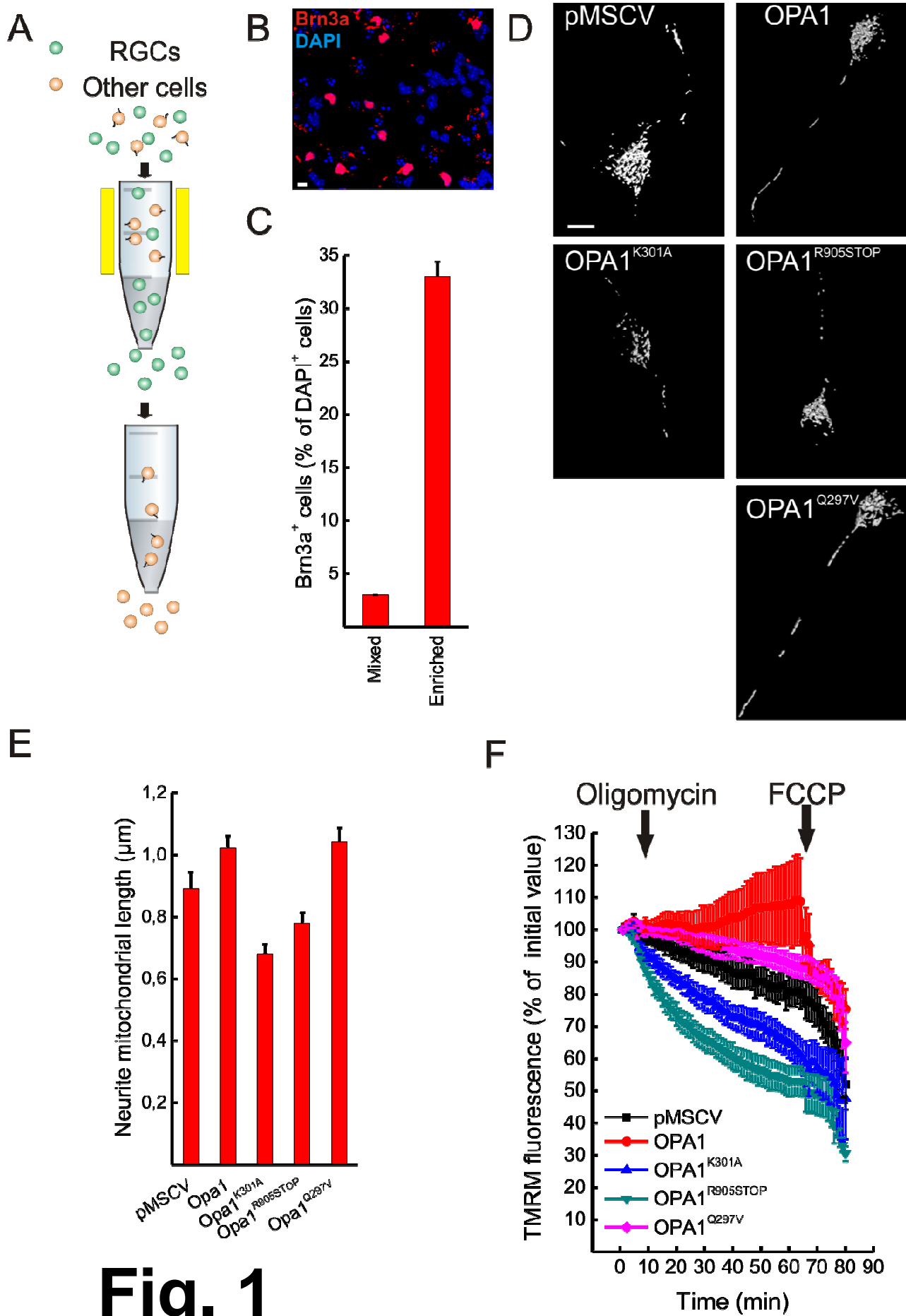


Fig. 1

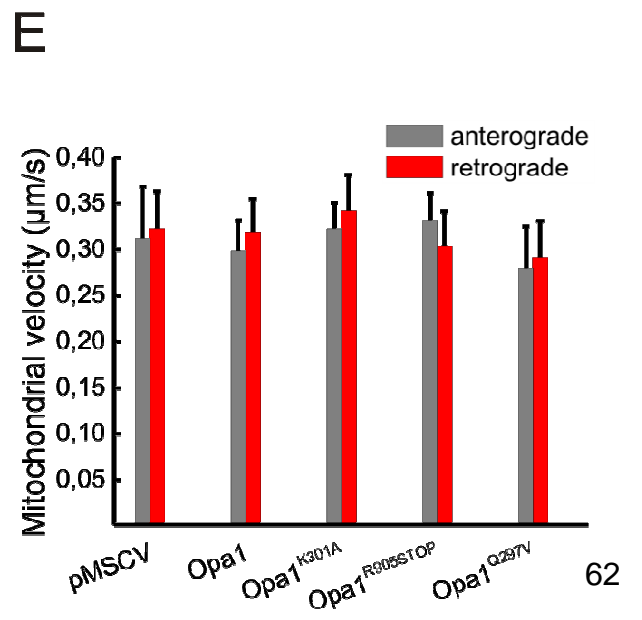
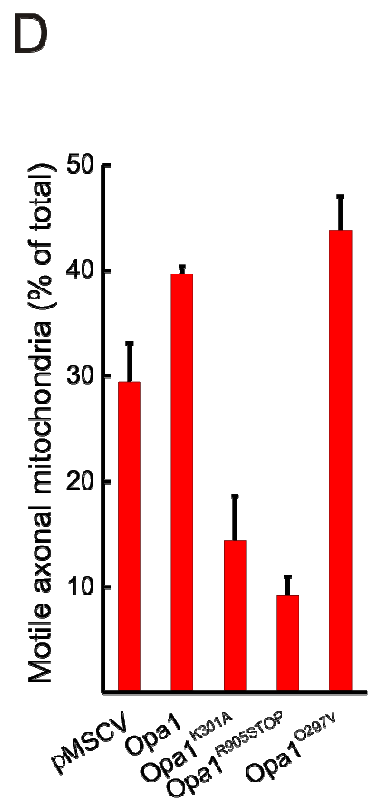
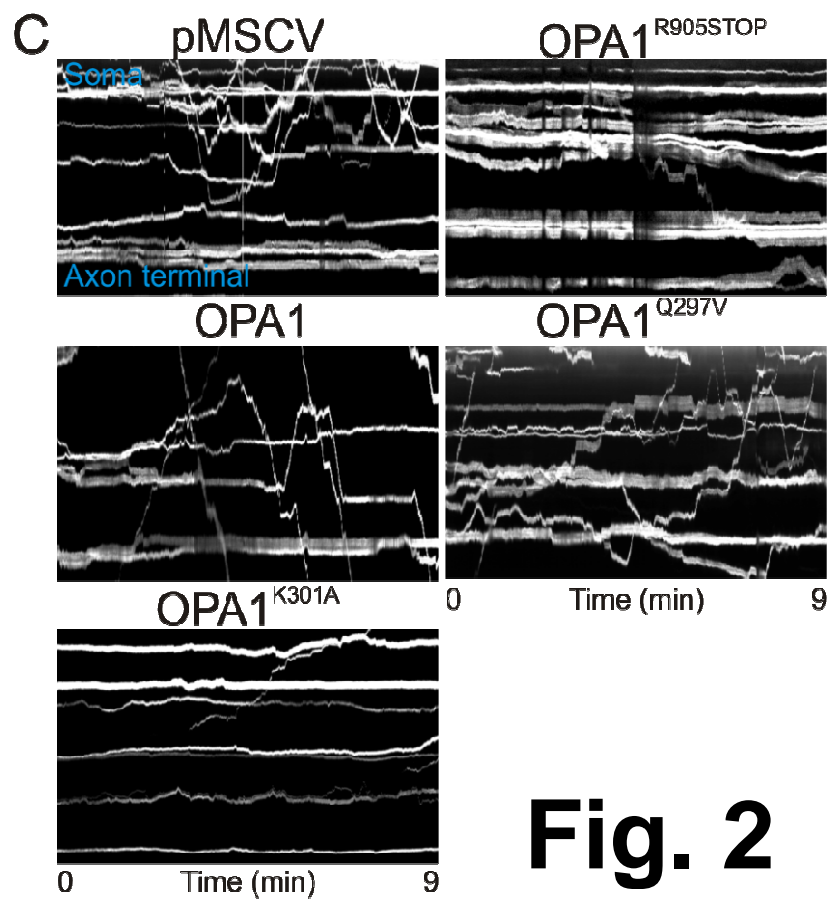
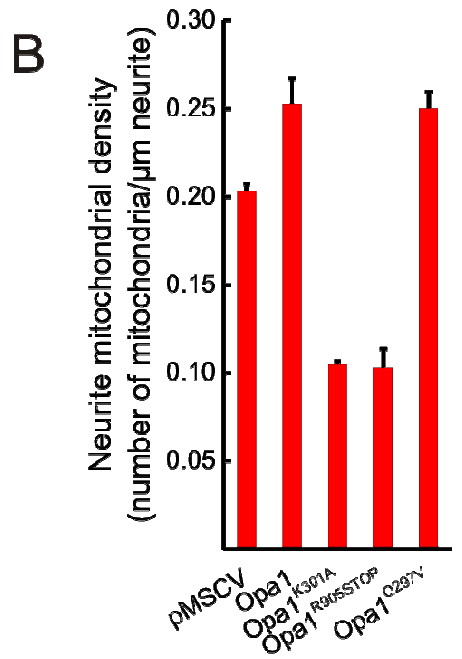
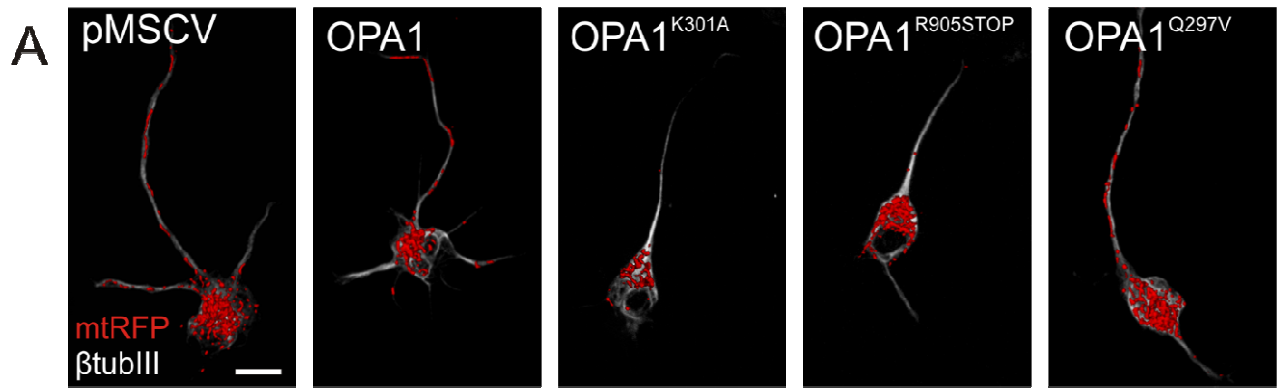


Fig. 2

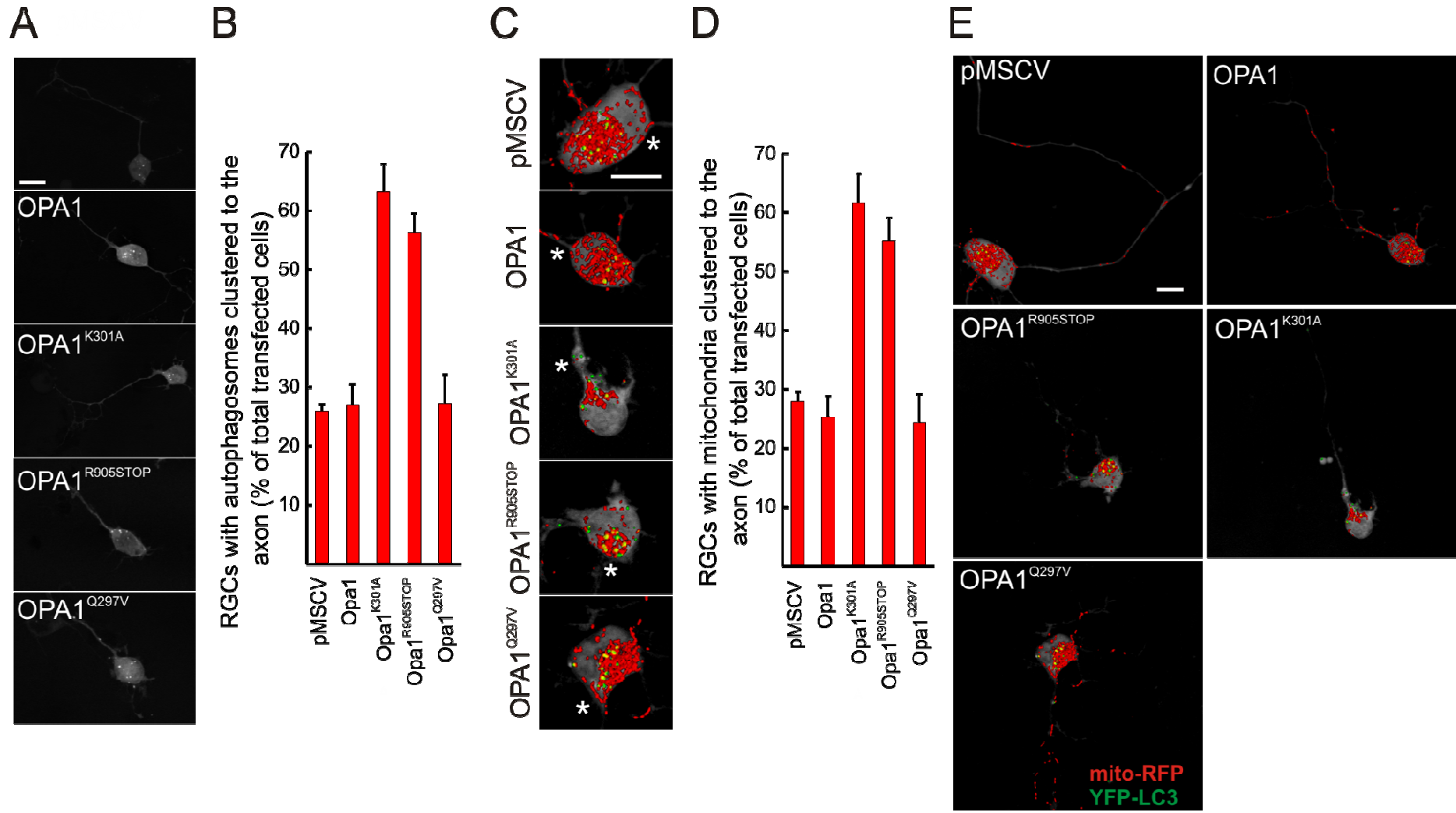


Fig. 3

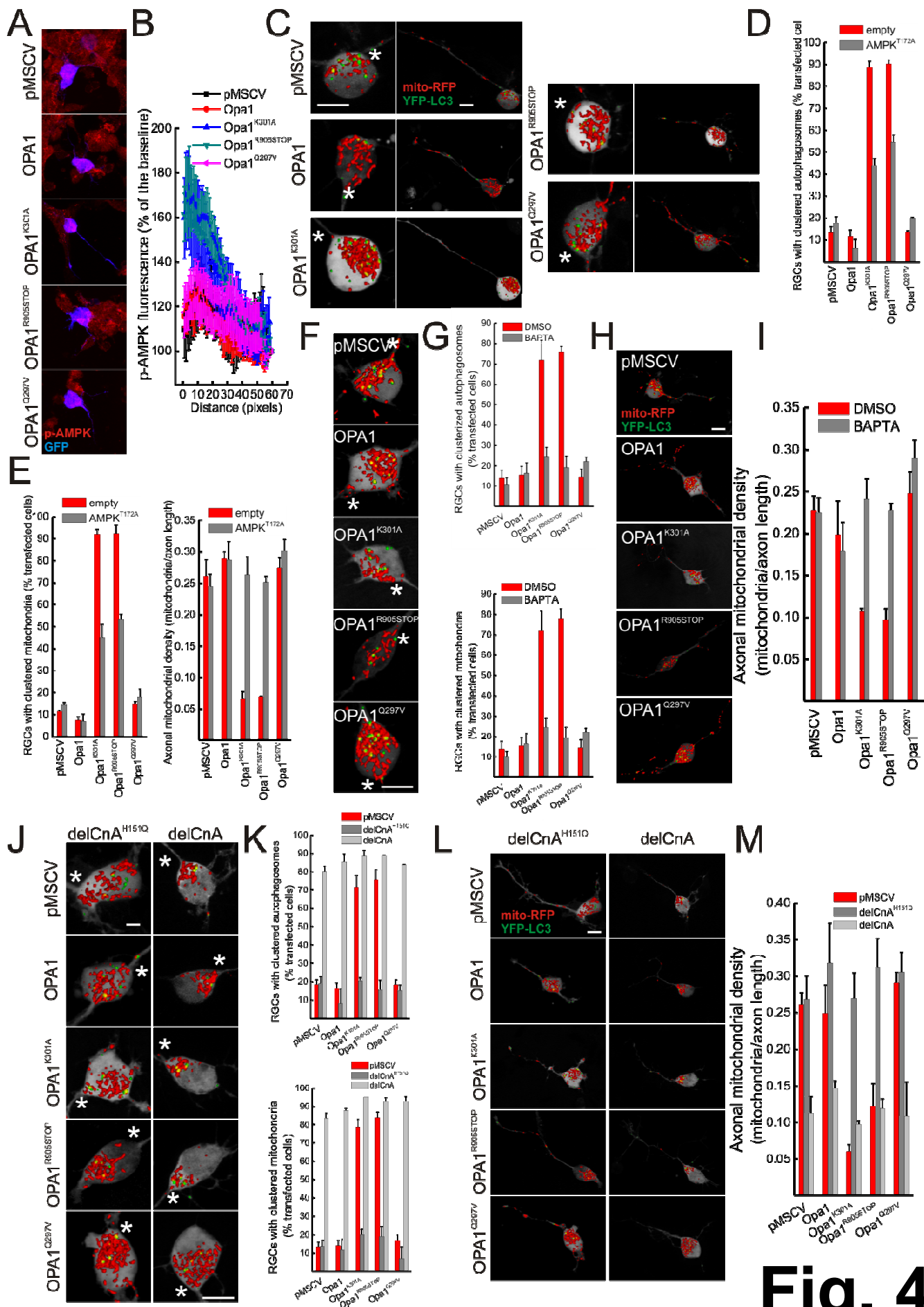


Fig. 4

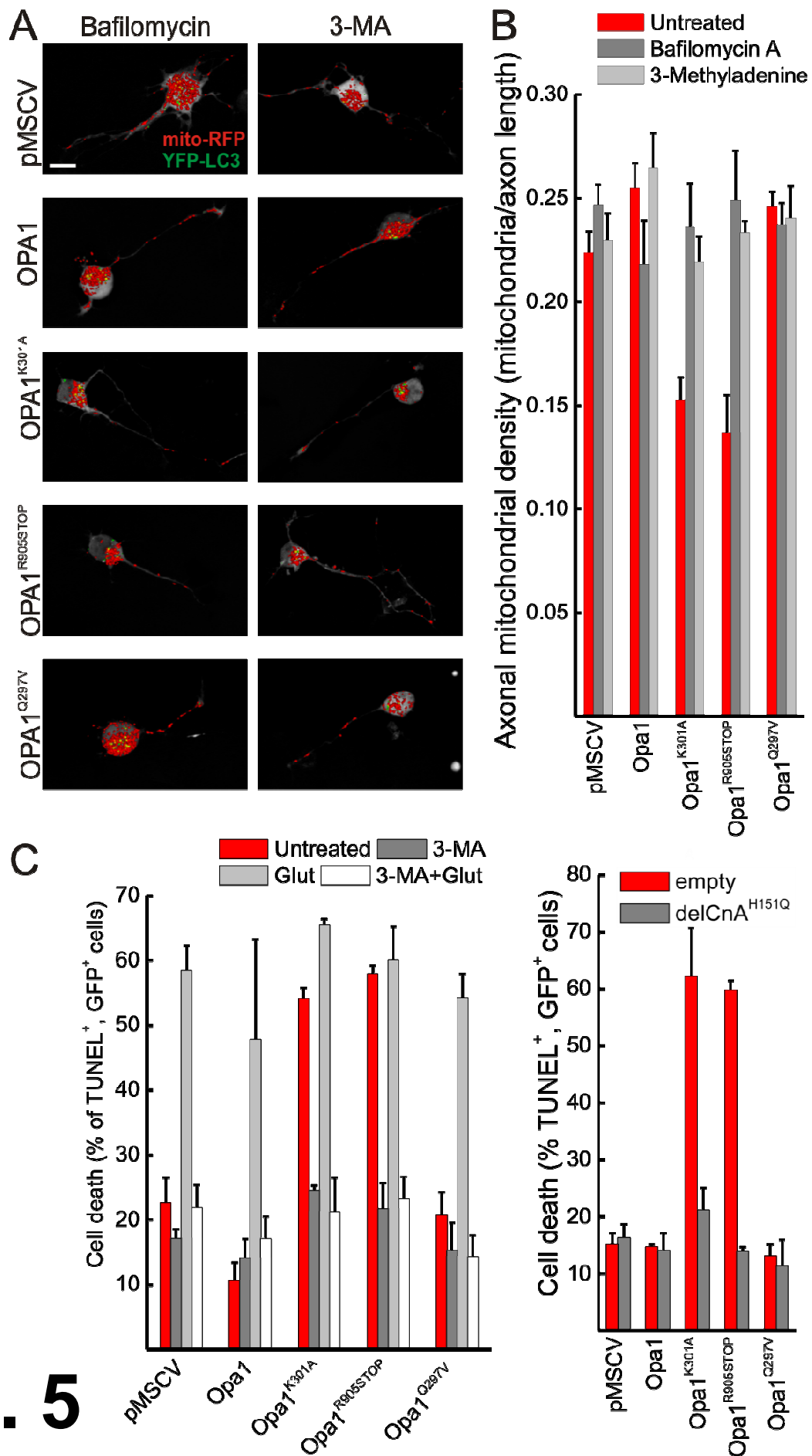


Fig. 5

A localized autophagic filter prevents entry of mitochondria carrying pathogenic Opa1 mutations in retinal ganglion cell axons

Marta Zaninello and Luca Scorrano

Supplementary Online Material

Supplementary online Figures

Figure S1. Opa1 pathogenic mutants fragment and depolarize mitochondria of primary RGCs harvested by immunopanning.

- (A)** Schematic representation of double-step immunopanning. Cells dissociated from P0 murine retinas are incubated on the first plate binds macrophages and endothelial cells (big orange circles). The supernatant contains Thy1⁻ cells (green circles) and RGCs (small orange circles), which are further bound to the selection plate.
- (B)** PCR products amplified from mouse cDNA obtained from RGCs harvested by immuno-panning. The further retinal cells markers were amplified: Brn3c (RGCs), Thy1 (RGCs, macrophages and endothelial cells), Iba1 (macrophages), Pecam1 (endothelial cells), GFAP (astrocytes), Lhx1 (horizontal cells), Stx1a (amacrine cells), Rcvr (photoreceptors) and Vsx2 (bipolar cells).
- (C)** Representative 3D reconstructions of stacks of confocal images of the fluorescence of mtRFP in primary RGCs cotransfected with the indicated plasmids and fixed after 24 hours. 70 confocal images of mtRFP fluorescence were acquired along the z-axis, deconvolved and 3D reconstructed (mtRFP) using the appropriate plugins of ImageJ. Bar, 20 μ m.

Figure S2. Opa1 pathogenic mutants deplete mitochondria from RGCs axons and reduces dendritic spines.

- (A)** Representative 3D reconstructions of stacks of confocal images of the fluorescence of mtRFP (red), SMI-312 (green) and GFP (blue) in primary RGCs cotransfected with the indicated plasmids. Bar, 20 μ m.
- (B)** Representative z-projects of stacks of confocal images of the fluorescence of GFP in primary RGCs cotransfected with the indicated plasmids. Bar, 20 μ m.
- (C)** Quantitative analysis of the spine density in cellular processes of RGCs cotransfected with GFP and the indicated plasmids. Data represent average \pm SEM of 4 independent experiments.

Figure S3. Mitochondria expressing Opa1 pathogenic mutants present hallmarks of mitophagy.

- (A)** Representative 3D reconstructions of stacks of confocal images of the fluorescence of mtRFP (red) and YFP-LC3 (green) in primary RGCs cotransfected with the indicated

plasmids. Bar, 20 μm .

(B) Quantitative analysis of autophagosomes and mitochondrial colocalization using Manders' coefficient (min colocalization = 0; max colocalization = 1). Data represent average \pm SEM of 6 independent experiments.

(C) Analysis of cells presenting Parkin surrounding mitochondria. The number of Parkin positive RGCs is normalized to the number of total transfected RGCs. Data represent the average \pm SEM of 2 independent experiments.

(D) Representative 3D reconstructions of stacks of confocal images of the fluorescence of mtRFP (red) and YFP-Parkin (green, translocated to mitochondria; grey, cytosolic) in primary RGCs cotransfected with the indicated plasmids. Bar, 20 μm .

Figure S4. Calcineurin do not alter autophagic flux in RGCs expressing Opa1 pathogenic mutants.

(A) Analysis of the autophagic flux in RGCs expressing the indicated plasmids and treated with Bafilomycin. Data represent the average \pm SEM of 2 independent experiments.

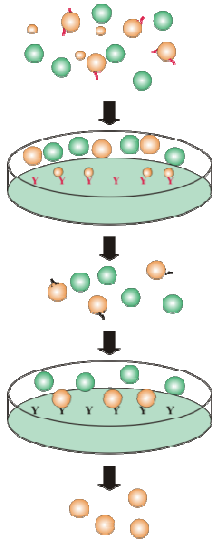
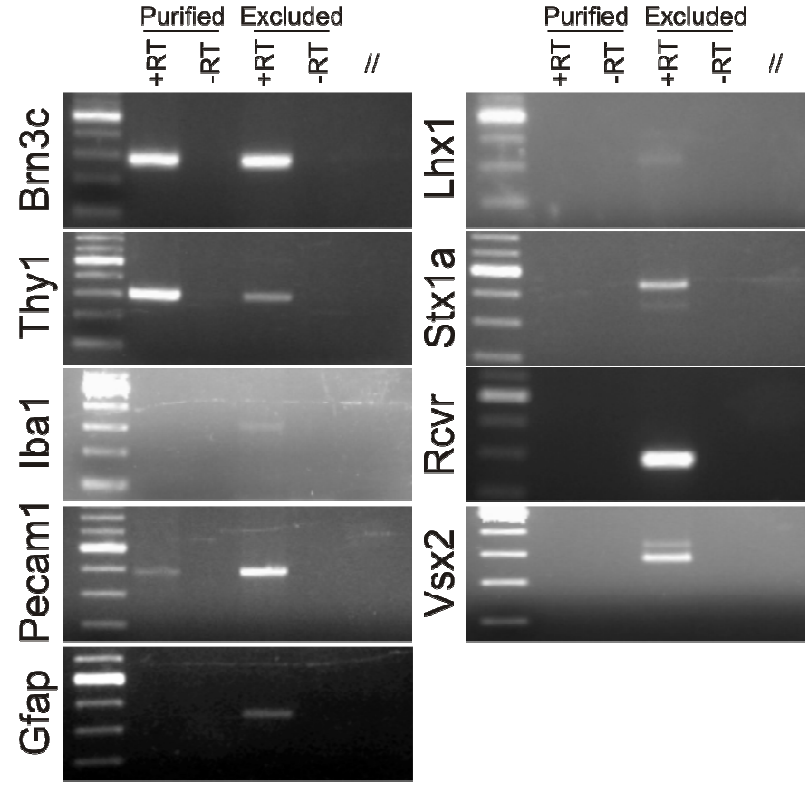
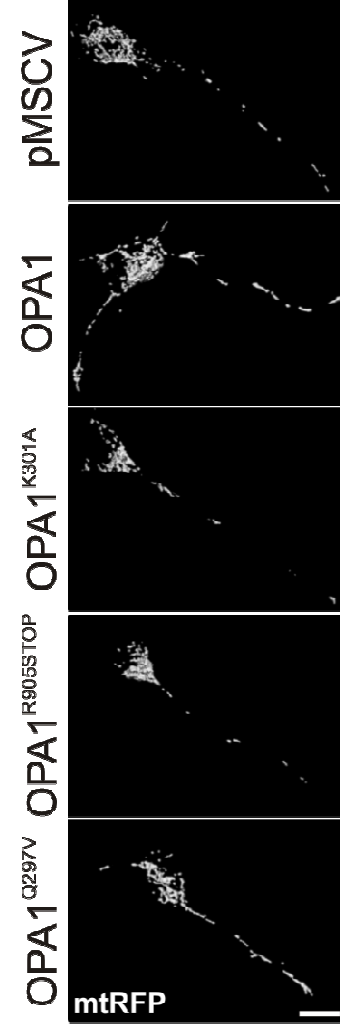
Figure S5. The blockage of autophagy do not rescue autophagosome and mitochondria distribution in the soma.

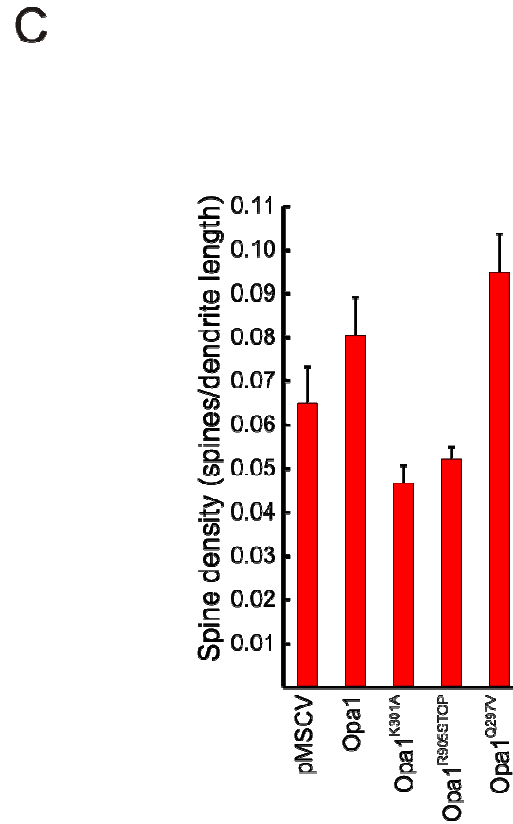
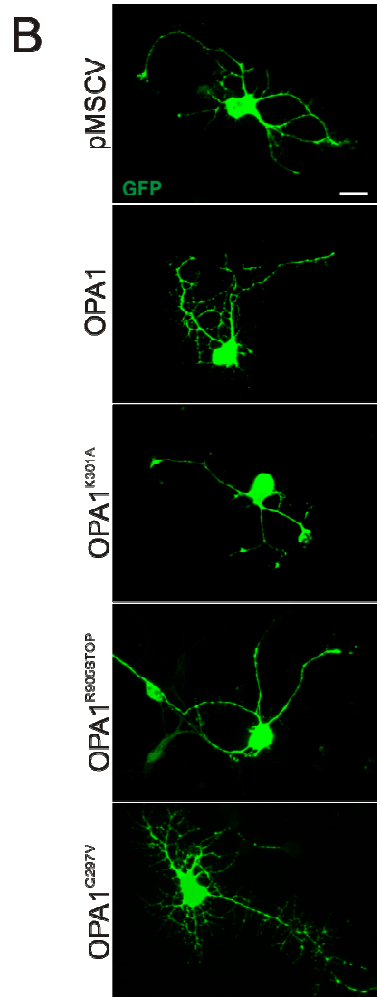
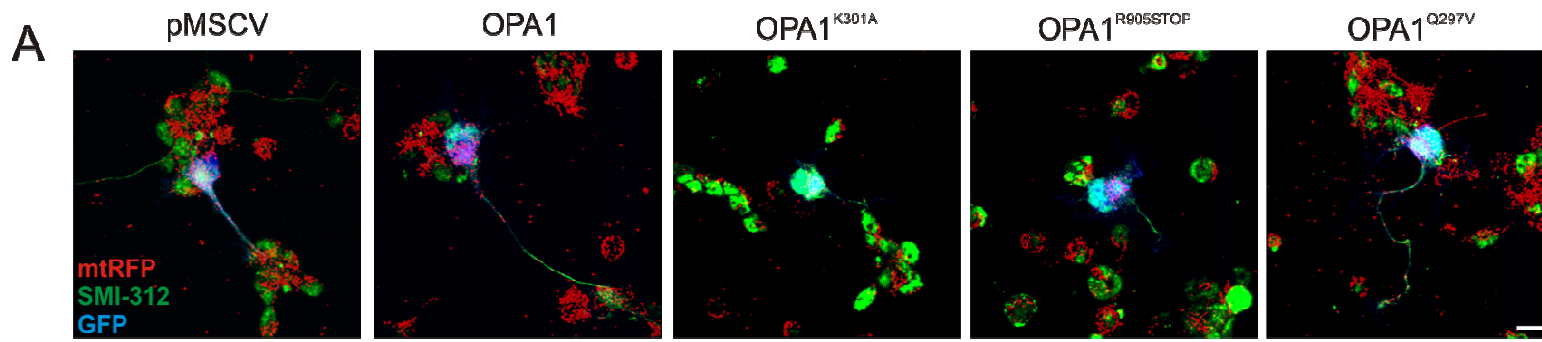
(A) Representative 3D reconstructions of stacks of confocal images of the fluorescence of mtRFP (red) and YFP-LC3 (green) in the soma of primary RGCs cotransfected with the indicated plasmids and treated with Bafilomycin or 3-Methyladenine. The axon is indicated by an asterisk. Bar, 20 μm .

(B) Quantitative analysis of autophagosomes and mitochondria accumulation in the axonal hillock in RGCs cotransfected with YFP-LC3, mtRFP and the indicated plasmids. Data represent average \pm SEM of 4 independent experiments.

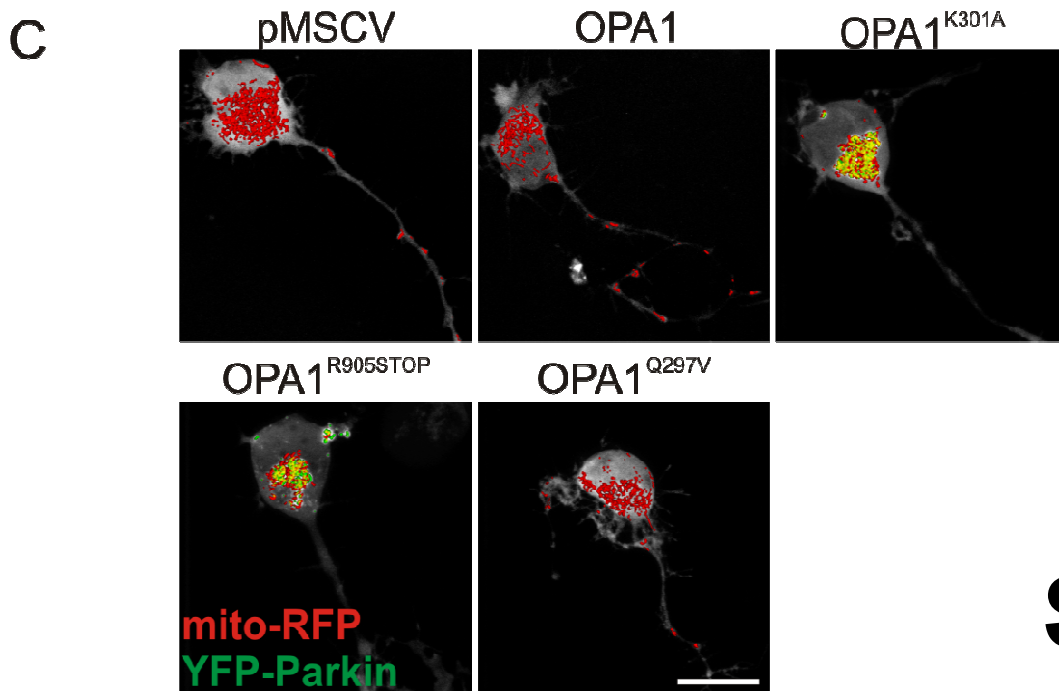
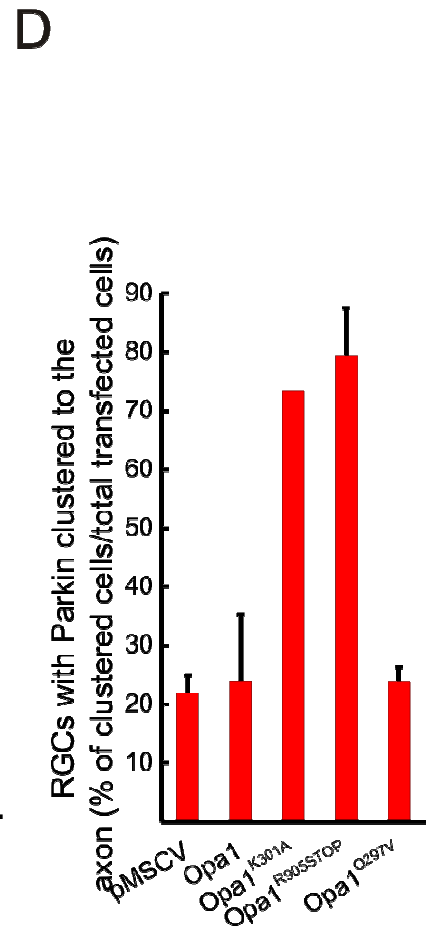
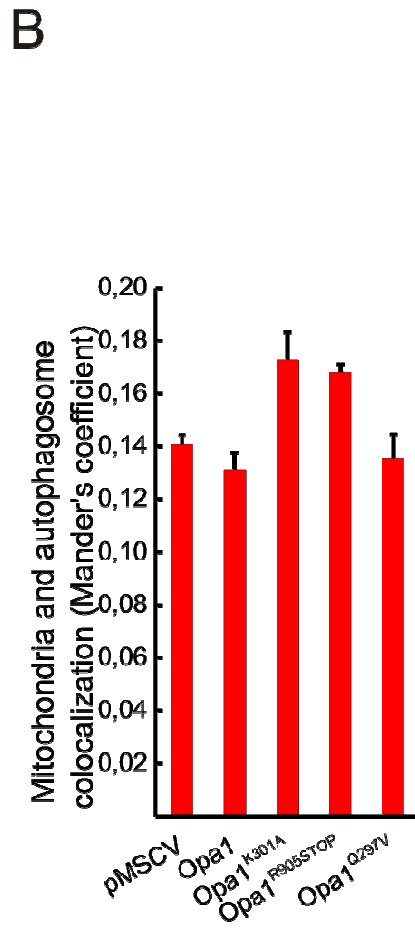
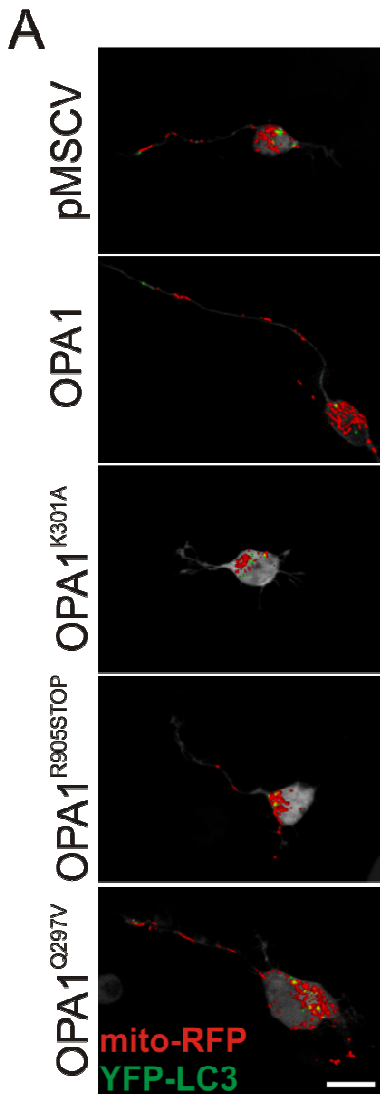
A

- α-macrophage antiserum
- α-Thy1 antibody
- RGCs
- Thy1+ cells
- Thy1- cells

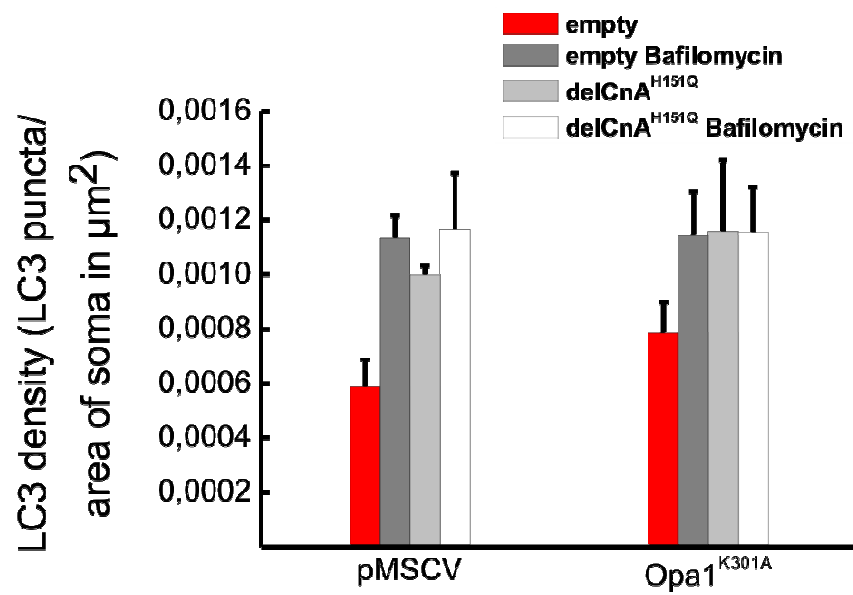
**S1****B****C**



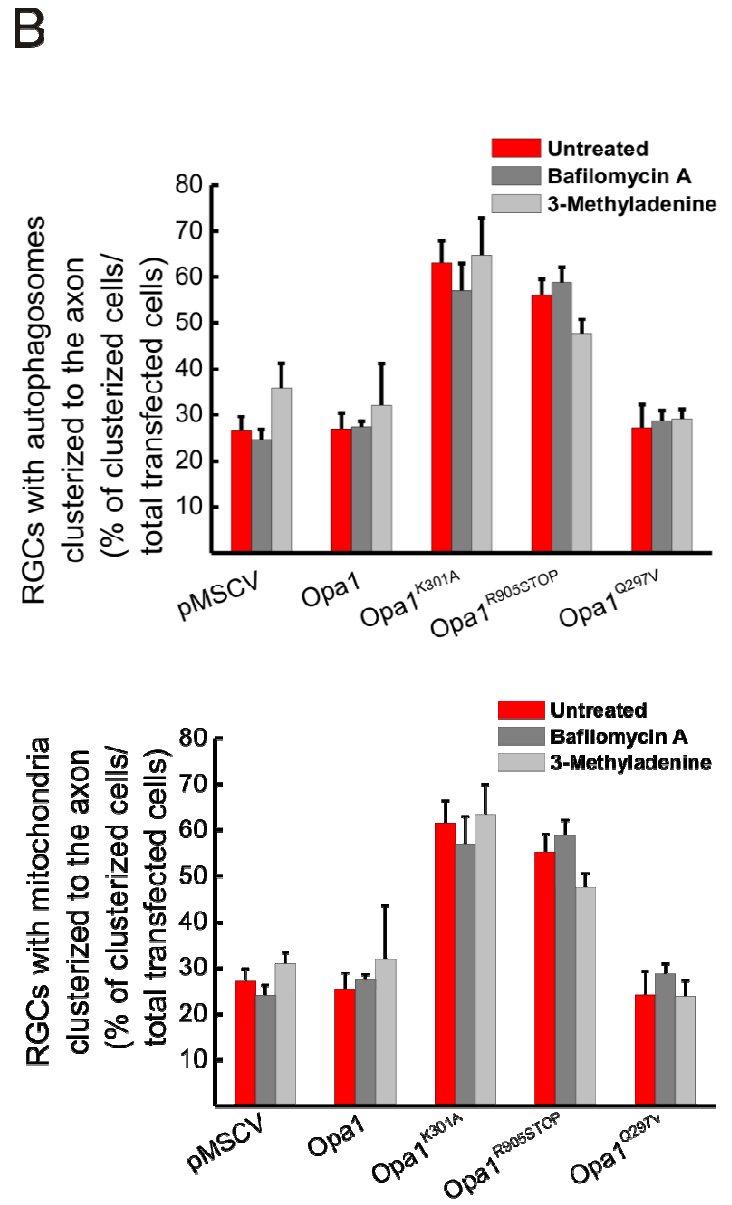
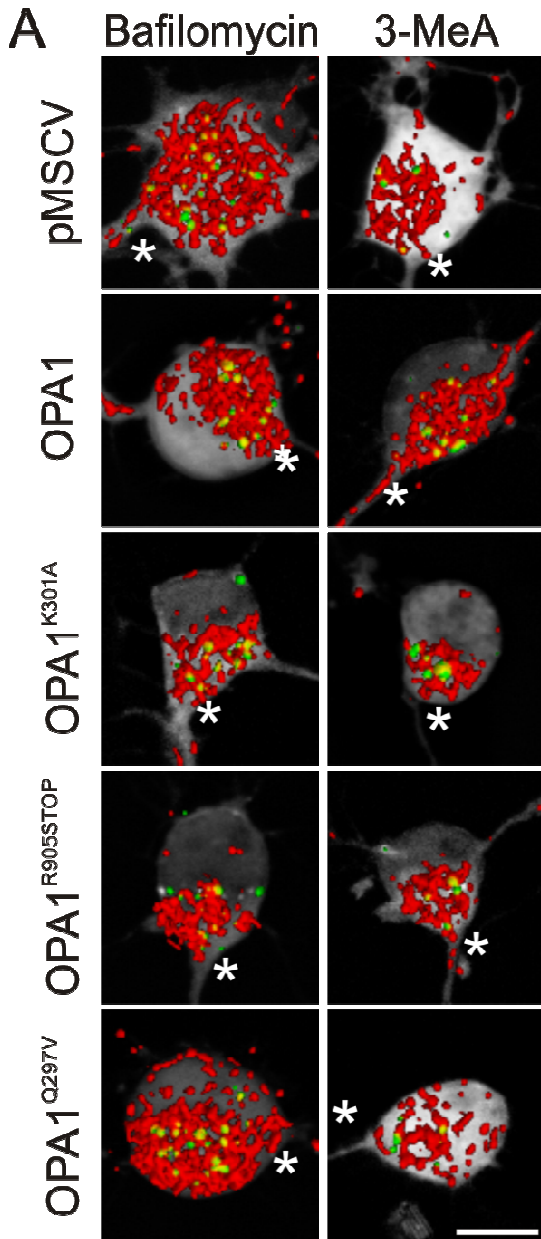
S2



S3



S4



S5

5. Future perspectives

Our *in vitro* data suggest that inhibition of autophagy could be a feasible therapeutic strategy to treat ADOA. However, we need to test if in a mouse model autophagosomes accumulate in the axonal hillock together with mitochondria that are excluded from the axon, and whether the progressive blindness is blunted by autophagy inhibition. Since the currently available mouse models of ADOA develop blindness in advanced age, we decided to develop a different model based on the conditional ablation of Opa1 in the retinal ganglion cells. This mouse model will be instrumental in testing the efficacy of autophagy inhibition to treat ADOA, but it requires first to be characterized. We therefore present here an account of our initial characterization of the conditional Opa1 ablation in RGCs.

Introduction

Autosomal dominant optic atrophy (ADOA) is the most common form of inherited optic neuropathy (Alexander et al., 2000a;Delettre et al., 2000a). ADOA onset is usually during childhood and it is characterized by the slower, progressive and bilateral decrease in visual acuity, tritanopia, sensitivity loss in the central visual fields, and pallor of the optic nerve (Ferre et al., 2005;Votruba et al., 1997). ADOA presents a large variability in the severity of clinical expression. Indeed, the same molecular defect may result in non-penetrant unaffected cases or in very severe, early onset cases (Carelli et al., 2004;Ferre et al., 2005).

Histopathological analysis in post-mortem ADOA patients reveal the selective loss of retinal ganglion cells (RGCs) with a prevalence in the central retina and optic atrophy, characterized by loss of myelin and nerve tissue (Johnston et al., 1979;Kjer et al., 1983). Linkage studies identifies three genes associated to ADOA (Alexander et al., 2000a;Delettre et al., 2000a) and the 75% of ADOA patients show mutations in Optic Atrophy 1 (Opa1) (Lenaers et al., 2012). Opa1 is ubiquitously expressed throughout the body: in the heart, skeletal muscle, liver, testis, brain and retina (Alexander et al., 2000a). Moreover, OPA1 is not more abundant in RGCs than in other retinal cells (Kamei et al., 2005). Thus, OPA1 levels do not explain the selective loss of RGCs in ADOA.

Three ADOA mouse models have been generated carrying the most frequent mutations observed in patients. In the mouse model described by Alavi and co-workers (2007), a STOP codon is inserted in position 285, truncating the protein at the beginning of the GTPase domain. In the second model, an in-frame splice site mutation (329-355del) deletes 27 amino acids in the GTPase domain (Davies et al., 2007). Finally, a frame shift mutation (delTTAG) generates a protein lacking 58 amino acids (Sarzi et al., 2012). The mutations result in 30% to 50% reduction of OPA1 levels in the heterozygous animal, whereas homozygous mutants are embryonically lethal at E8.5, E13.5 and E10.5. The first two models of Opa1 display minimal retinal defects and normal myelination until 24 months and start to decrease visual function and visual evoked potentials (VEP) amplitude at 20 and 12 months, respectively (Alavi et al., 2007;Davies et al., 2007;Barnard et al., 2011). Morphologically, the only difference is reported in dendritic atrophy at 10 months (Williams et al., 2012). The Opa1^{delTTAG} mutant decrease VEP amplitude at 9 months and shows a significant axonal degeneration and demyelination at 16 months, starting from 5 months. Interestingly, electron microscopy reveals increased autophagic vesicles in optic

and sciatic nerves (Sarzi et al., 2012). Moreover, the same mouse model present mitochondrial abnormalities in heart and muscles.

However, these mouse models of ADOA do not show RGCs loss not follow the early onset of the disease, reducing their usefulness to investigate ADOA pathogenesis and most importantly treatment. We therefore set out to generate and characterize a mouse model where Opa1 is specifically ablated in a subset of RGCs.

Results

Opa1^{flx/flx} mice were generated following the strategy depicted in Fig. 1A and described in (Cogliati et al., 2013). In order to delete Opa1 specifically in a subset of RGCs, we crossed Opa1^{flx/flx} mice with a Grik4-Cre line, which expresses the recombinase in a subset of RGCs (Ivanova et al., 2010). Mice were viable and fertile and did not show any defect in growth (Fig.1C). Moreover, histology was not altered in the whole body (data not shown) or cerebral districts where the Grik4 promoter has been reported to be active (Fig.1D). Histological studies revealed a slight reduction in Opa1^{flx/flx} RGCs number at 6 months as compared to Opa1^{flx/-} RGCs (Fig.2A), but the diameter or in the density of axons, measured by radial distribution of neurofilament intensity (Fig.2B,2C). Visual acuity was reduced already by 50% in 4-months old but not in 3 months old Opa1^{flx/flx} mice at every tested spatial and temporal frequency (Fig.2D).

Discussion

We generated an Opa1 conditional knock-out mice in a subset of RGCs. Opa1^{flx/flx} mice impaired visual sight much earlier than ADOA mouse models present so far (Alavi et al., 2007; Davies et al., 2007; Barnard et al., 2011), representing a model more adequate to follow the early onset of the disease in humans. Where Opa1 is supposed to be ablated, that is, in RGCs, cerebellum and in CA3 area of the hippocampus, we did not detect apoptosis or necrosis, suggesting that mechanisms responsible for ADOA are much more complex than simple cell death. Moreover, the impressive loss of sight observed in Opa1^{flx/flx} mice depends on a minor population of RGCs. This suggests the great impact of Opa1-dependent mitochondrial dysfunction on RGCs functioning, and how this may influence the overall RGCs network, since gap junctions in the retinal axons have been described. Gap junctions are responsible for the centripetal extension of spreading neuron death, allowing the passage of pro-apoptotic signals like calcium and ROS (Rawanduzy et al., 1997). Thus, even if the characterization is not complete, preliminary data suggest the

great potential of this new ADOA mouse model that, in turn, may be a useful tool to investigate possible treatments for the disease.

Experimental procedures

Animal generations

Opa1^{flx/flx} mice were described in (Cogliati et al., 2013) following the strategy depicted in Fig.1A. Opa1^{flx/flx} mice were crossed with Grik4-Cre mice (Jackson laboratories). Genotypes were assessed using tail genomic DNA and the following primers. For Opa1^{flx/flx}: PRIMER Ck1 5'-CAG TGT TGA TGA CAG CTC AG-3'; PRIMER Ck2 5'-CAT CAC ACA CTA GCT TAC ATT TGC-3'; for Cre: PRIMER forward 5'- GCG GTC TGG CAG TAA AAA CTA TC-3'; PRIMER reverse 5'- GTG AAA CAG CAT TGC TGT CAC TT-3'. All mice procedures were performed according to protocols approved by the local Ethic committees (protocol 32/2011 CEASA University of Padova, Venetian Institute of Molecular Medicine).

Histology

Animals were anesthetized and perfused with *paraformaldehyde 4% (w/v) (Sigma)*. *Eyes, cerebelli and hippocampi were dissected, included in paraffin and cut with 0.5 μm sections using ultramicrotome (Leica)*. *Section were stained using haematoxylin and eosin (Sigma) or with anti-neurofilament200 antibody 1:500 (Sigma)*.

Number of axons in the optic nerve was assessed by measured by radial distribution which measures average signal intensity of neurofilament along an arc of the circle at various radii.

Visual acuity

Visual acuity was measured using the AgorsOptokinetic Drum (Instead technologies), following manufacturer's instructions using spatial and temporal frequencies listed in Fig.2D.

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Figure legends

Figure 1. *Opa1* ablation in cerebral districts do not alter mouse growth or cerebral histology.

(A) Schematic diagram of conditional targeting constructs for *Opa1*. The 5' UTR, exons (black boxes), LoxP sites (white arrows), FRT recombination sites, and PGK-neomycin cassette (white box) are indicated. The locations of PCR primers (*ck1* = primer check1 forward, *ck2* = primer check2 reverse) are indicated. Dimensions are not in scale. $Opa1^{flx/flx}$, $Opa1^{flx/-}$ and, $Opa1^{-/-}$ indicate homozygous and heterozygous for the floxed allele, and $Opa1^{wt}$ gene, respectively.

(B) PCR analysis of tail genomic DNA showing the genotypes of the mice. *Opa1* is amplified at 700 bp and *Opa1* floxed sequence shifts the band at 800 bp (left panel). *Cre* is amplified at 100 bp (right panel).

(C) Quantification of mouse weight in $OPA1^{flx/flx}Cre^{+/-}$ and $OPA1^{flx/-}Cre^{+/-}$ mice classified per sex. Data represent average \pm SEM of 7 mice.

(D) Representative haematoxylin and eosin staining of transversal sections of cerebellum and hippocampus from $OPA1^{flx/flx}Cre^{+/-}$ and $OPA1^{flx/-}Cre^{+/-}$ mice. 4 months-old mice were sacrificed, eyes dissected and fixed. After fixation and inclusion in paraffin, eyes were sectioned and stained

Figure 2. Opa1 ablation in a subset of RGCs does not alter retinal and optic nerve but impairs visual acuity .

(A) Representative haematoxylin and eosin staining of transversal sections of retinas from OPA1^{flx/flx}Cre^{+/-} and OPA1^{flx/-}Cre^{+/-} mice. 4 months-old mice were sacrificed, eyes dissected and fixed. After fixation and inclusion in paraffin, eyes were sectioned and stained.

(B) Representative neurofilament staining (left) and enlargements (right) of transversal sections of optic nerves from OPA1^{flx/flx}Cre^{+/-} and OPA1^{flx/-}Cre^{+/-} mice. 6 months-old mice were sacrificed, eyes dissected and fixed. After fixation and inclusion in paraffin, optic nerves were sectioned and immunostained.

(C) Quantitative analysis of radial distribution of neurofilament in cross-sectional areas of optic nerves of 6 months-old OPA1^{flx/flx}Cre^{+/-} and OPA1^{flx/-}Cre^{+/-} mice. Data represent average \pm SEM of 3 mice.

(D) Quantitative analysis of visual acuity in 3 (left) and 4 (right) months-old OPA1^{flx/flx}Cre^{+/-} and OPA1^{flx/-}Cre^{+/-} mice using the optokinetic drum device. Mice were subjected to visual stimuli with temporal and spatial frequencies indicated. Visual acuity is proportional to the percentage of correct answers to the stimulus. Data represent average \pm SEM of 6 mice.

Reference List

Alavi MV, Bette S, Schimpf S, Schuettauf F, Schraermeyer U, Wehrl HF, Ruttiger L, Beck SC, Tonagel F, Pichler BJ, Knipper M, Peters T, Laufs J, Wissinger B (2007) A splice site mutation in the murine Opa1 gene features pathology of autosomal dominant optic atrophy. *Brain* 130:1029-1042.

Alexander C, Votruba M, Pesch UE, Thiselton DL, Mayer S, Moore A, Rodriguez M, Kellner U, Leo-Kottler B, Auburger G, Bhattacharya SS, Wissinger B (2000) OPA1, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28. *Nat Genet* 26:211-215.

Barnard AR, Charbel IP, Perganta G, Williams PA, Davies VJ, Sekaran S, Votruba M, MacLaren RE (2011) Specific deficits in visual electrophysiology in a mouse model of dominant optic atrophy. *Exp Eye Res* 93:771-777.

Carelli V, Ross-Cisneros FN, Sadun AA (2004) Mitochondrial dysfunction as a cause of optic neuropathies. *Prog Retin Eye Res* 23:53-89.

Cogliati S, Frezza C, Soriano ME, Varanita T, Quintana-Cabrera R, Corrado M, Cipolat S, Costa V, Casarin A, Gomes LC, Perales-Clemente E, Salviati L, Fernandez-Silva P, Enriquez JA, Scorrano L (2013) Mitochondrial cristae shape determines respiratory chain supercomplexes assembly and respiratory efficiency. *Cell* 155:160-171.

Davies VJ, Hollins AJ, Piechota MJ, Yip W, Davies JR, White KE, Nicols PP, Boulton ME, Votruba M (2007) Opa1 deficiency in a mouse model of autosomal dominant optic atrophy impairs mitochondrial morphology, optic nerve structure and visual function. *Hum Mol Genet* 16:1307-1318.

Delettre C, Lenaers G, Griffoin JM, Gigarel N, Lorenzo C, Belenguer P, Pelloquin L, Grosgeorge J, Turc-Carel C, Perret E, Astarie-Dequeker C, Lasquelléc L, Arnaud B, Ducommun B, Kaplan J, Hamel CP (2000) Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. *Nat Genet* 26:207-210.

- Ferre M, Amati-Bonneau P, Tourmen Y, Malthiery Y, Reynier P (2005) eOPA1: an online database for OPA1 mutations. *Hum Mutat* 25:423-428.
- Ivanova E, Hwang GS, Pan ZH (2010) Characterization of transgenic mouse lines expressing Cre recombinase in the retina. *Neuroscience* 165:233-243.
- Johnston PB, Gaster RN, Smith VC, Tripathi RC (1979) A clinicopathologic study of autosomal dominant optic atrophy. *Am J Ophthalmol* 88:868-875.
- Kamei S, Chen-Kuo-Chang M, Cazevieuille C, Lenaers G, Olichon A, Belenguer P, Roussignol G, Renard N, Eybalin M, Michelin A, Delettre C, Brabet P, Hamel CP (2005) Expression of the Opa1 mitochondrial protein in retinal ganglion cells: its downregulation causes aggregation of the mitochondrial network. *Invest Ophthalmol Vis Sci* 46:4288-4294.
- Kjer P, Jensen OA, Klinken L (1983) Histopathology of eye, optic nerve and brain in a case of dominant optic atrophy. *Acta Ophthalmol (Copenh)* 61:300-312.
- Lenaers G, Hamel C, Delettre C, Amati-Bonneau P, Procaccio V, Bonneau D, Reynier P, Milea D (2012) Dominant optic atrophy. *Orphanet J Rare Dis* 7:46.
- Marchbank NJ, Craig JE, Leek JP, Toohey M, Churchill AJ, Markham AF, Mackey DA, Toomes C, Inglehearn CF (2002) Deletion of the OPA1 gene in a dominant optic atrophy family: evidence that haploinsufficiency is the cause of disease. *J Med Genet* 39:e47.
- Pesch UE, Leo-Kottler B, Mayer S, Jurklies B, Kellner U, Apfelstedt-Sylla E, Zrenner E, Alexander C, Wissinger B (2001) OPA1 mutations in patients with autosomal dominant optic atrophy and evidence for semi-dominant inheritance. *Hum Mol Genet* 10:1359-1368.
- Rawanduzy A, Hansen A, Hansen TW, Nedergaard M (1997) Effective reduction of infarct volume by gap junction blockade in a rodent model of stroke. *Neurosurg Focus* 2:E1.
- Sarzi E, Angebault C, Seveno M, Gueguen N, Chaix B, Bielicki G, Boddaert N, Mausset-Bonnefont AL, Cazevieuille C, Rigau V, Renou JP, Wang J, Delettre C, Brabet P, Puel JL, Hamel CP, Reynier P, Lenaers G (2012) The human OPA1delTTAG mutation induces premature age-related systemic neurodegeneration in mouse. *Brain* 135:3599-3613.
- Votruba M, Moore AT, Bhattacharya SS (1997) Genetic refinement of dominant optic atrophy (OPA1) locus to within a 2 cM interval of chromosome 3q. *J Med Genet* 34:117-121.
- Williams PA, Piechota M, von RC, Taylor E, Morgan JE, Votruba M (2012) Opa1 is essential for retinal ganglion cell synaptic architecture and connectivity. *Brain* 135:493-505.

Fig. 1

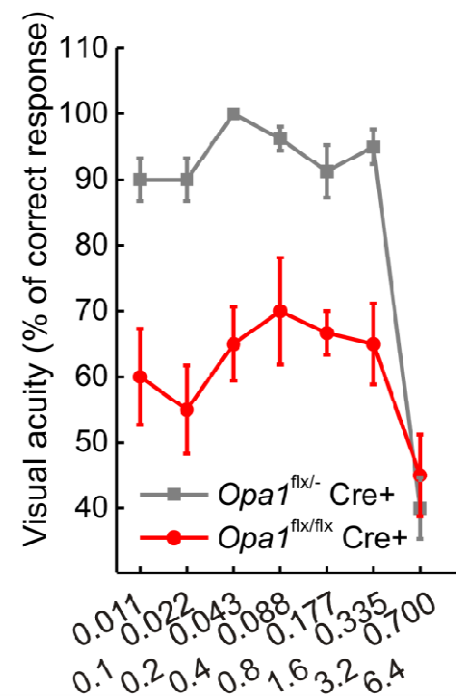
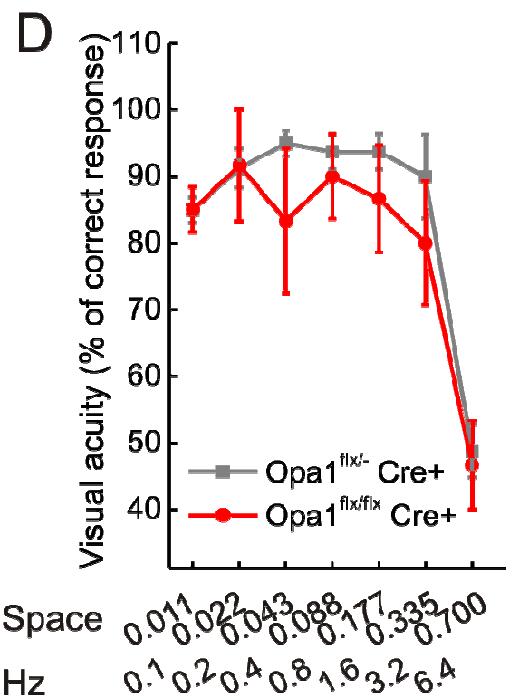
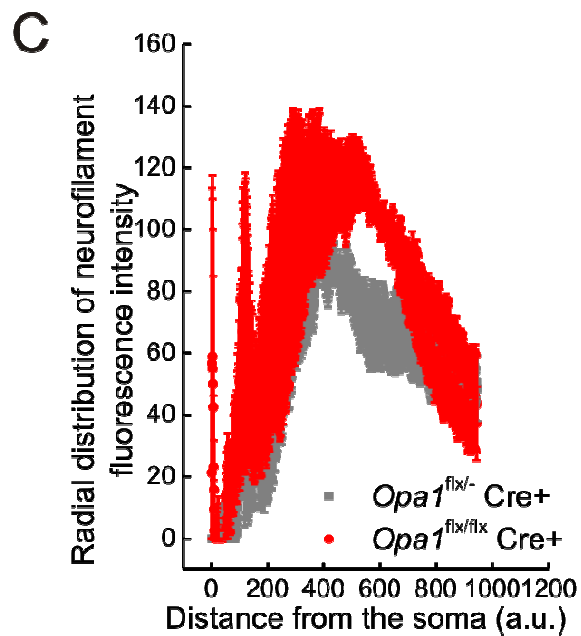
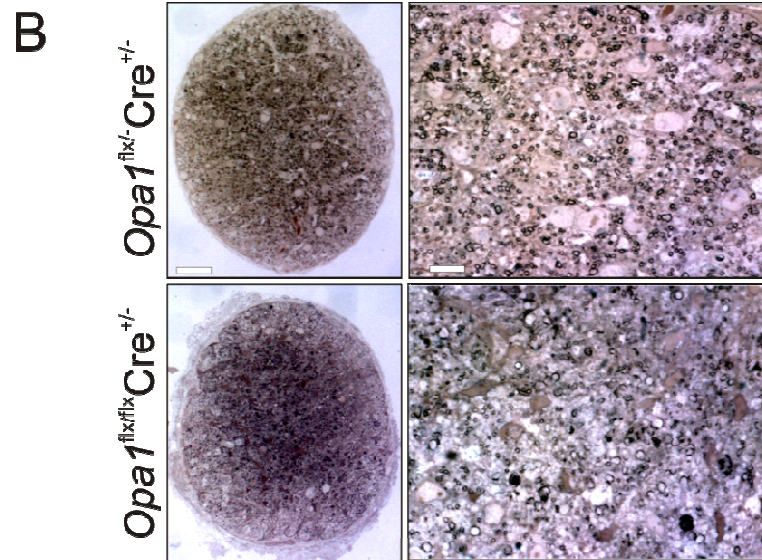
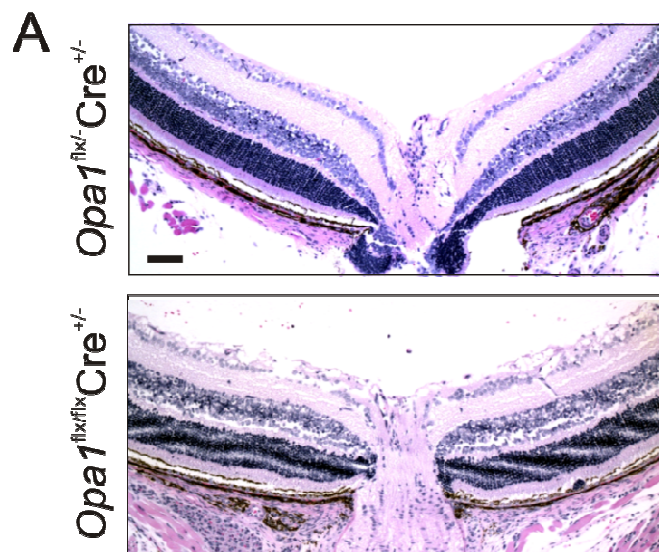
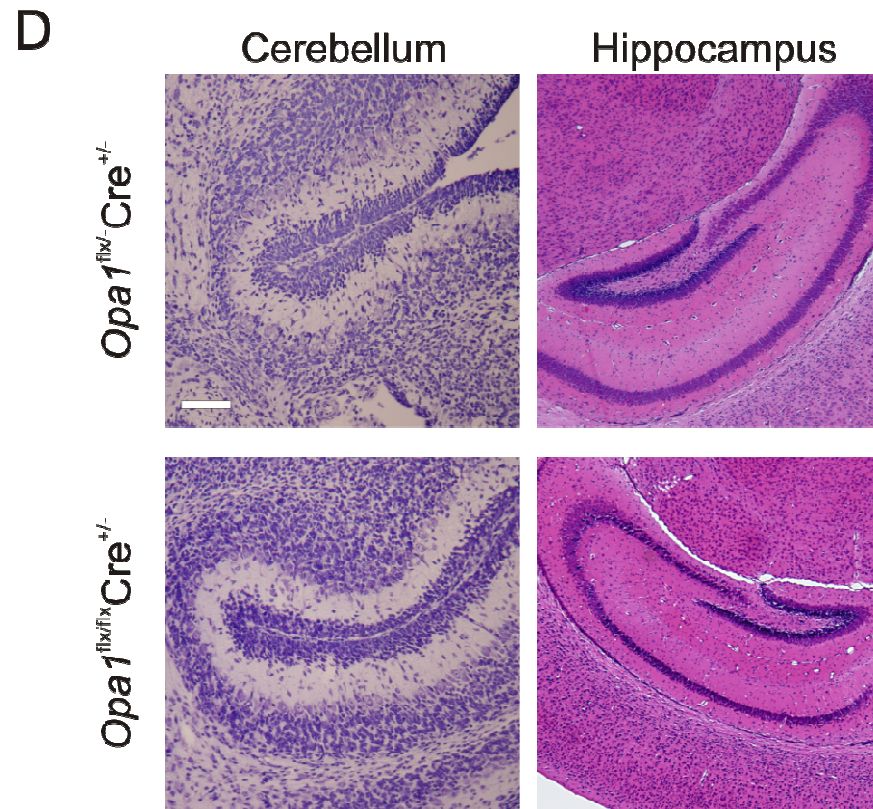
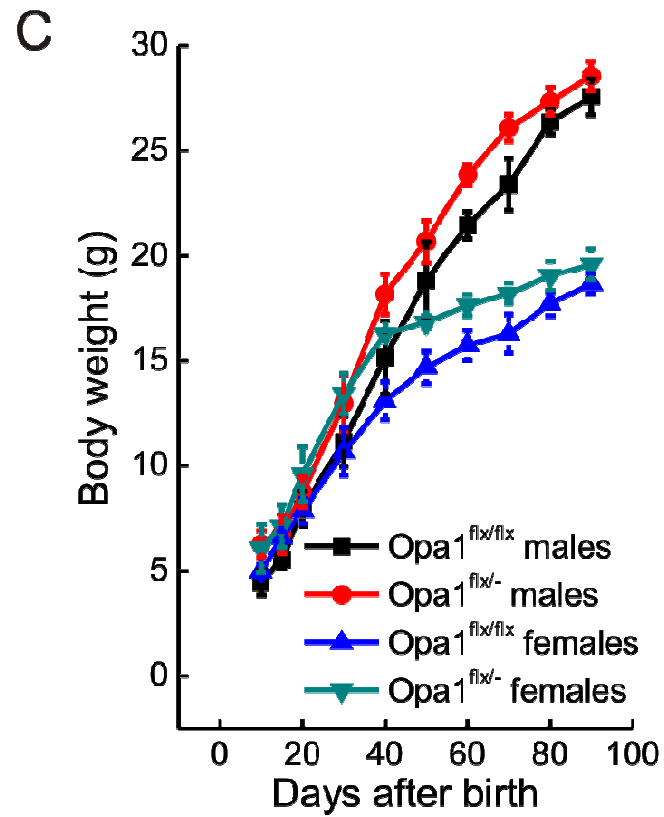
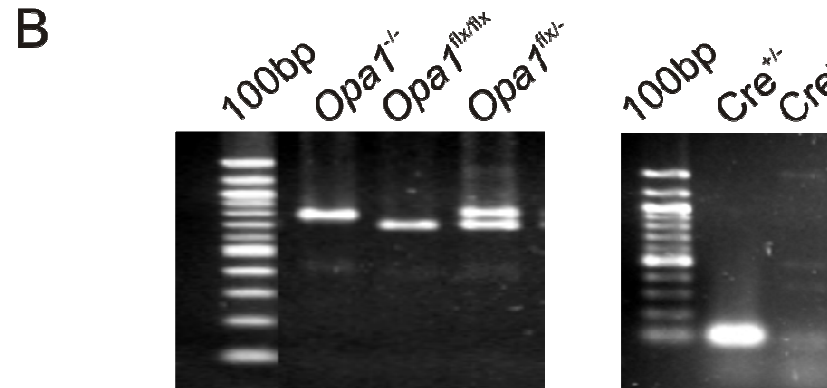
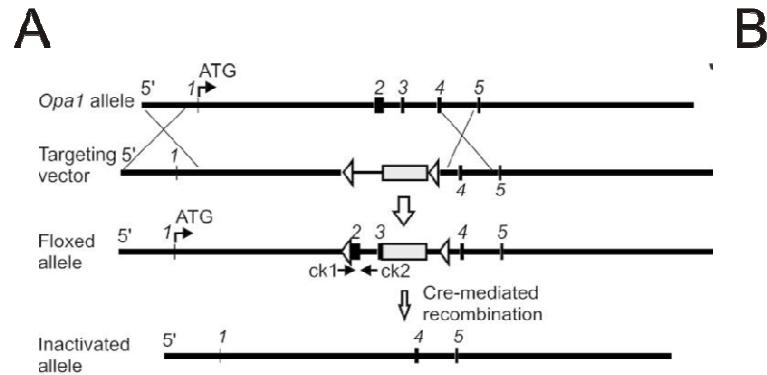


Fig. 2



6. Reference list

- Ahlskog JE (2009) Parkin and PINK1 parkinsonism may represent nigral mitochondrial cytopathies distinct from Lewy body Parkinson's disease. *Parkinsonism Relat Disord* 15:721-727.
- Alavi MV, Bette S, Schimpf S, Schuettauf F, Schraermeyer U, Wehr HF, Ruttiger L, Beck SC, Tonagel F, Pichler BJ, Knipper M, Peters T, Laufs J, Wissinger B (2007) A splice site mutation in the murine Opa1 gene features pathology of autosomal dominant optic atrophy. *Brain* 130:1029-1042.
- Alexander C, Votruba M, Pesch UE, Thiselton DL, Mayer S, Moore A, Rodriguez M, Kellner U, Leo-Kottler B, Auburger G, Bhattacharya SS, Wissinger B (2000a) OPA1, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28. *Nat Genet* 26:211-215.
- Alexander C, Votruba M, Pesch UE, Thiselton DL, Mayer S, Moore A, Rodriguez M, Kellner U, Leo-Kottler B, Auburger G, Bhattacharya SS, Wissinger B (2000b) OPA1, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28. *Nat Genet* 26:211-215.
- Amati-Bonneau P, Pasquier L, Lainey E, Ferre M, Odent S, Malthiery Y, Bonneau D, Reynier P (2005) Sporadic optic atrophy due to synonymous codon change altering mRNA splicing of OPA1. *Clin Genet* 67:102-103.
- Amato S, Liu X, Zheng B, Cantley L, Rakic P, Man HY (2011) AMP-activated protein kinase regulates neuronal polarization by interfering with PI 3-kinase localization. *Science* 332:247-251.
- Andrews H, White K, Thomson C, Edgar J, Bates D, Griffiths I, Turnbull D, Nichols P (2006) Increased axonal mitochondrial activity as an adaptation to myelin deficiency in the Shiverer mouse. *J Neurosci Res* 83:1533-1539.
- Arnoult D, Grodet A, Lee YJ, Estaquier J, Blackstone C (2005) Release of OPA1 during apoptosis participates in the rapid and complete release of cytochrome c and subsequent mitochondrial fragmentation. *J Biol Chem* 280:35742-35750.
- Ashrafi G, Schwarz TL (2013) The pathways of mitophagy for quality control and clearance of mitochondria. *Cell Death Differ* 20:31-42.
- Barnard AR, Charbel IP, Perganta G, Williams PA, Davies VJ, Sekaran S, Votruba M, MacLaren RE (2011) Specific deficits in visual electrophysiology in a mouse model of dominant optic atrophy. *Exp Eye Res* 93:771-777.
- Barres BA, Silverstein BE, Corey DP, Chun LL (1988) Immunological, morphological, and electrophysiological variation among retinal ganglion cells purified by panning. *Neuron* 1:791-803.
- Bateup HS, Takasaki KT, Saulnier JL, Deneffrio CL, Sabatini BL (2011) Loss of Tsc1 in vivo impairs hippocampal mGluR-LTD and increases excitatory synaptic function. *J Neurosci* 31:8862-8869.
- Baughman JM, Perocchi F, Girgis HS, Plovanich M, Belcher-Timme CA, Sancak Y, Bao XR, Strittmatter L, Goldberger O, Bogorad RL, Kotliansky V, Mootha VK (2011) Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter. *Nature* 476:341-345.
- Bereiter-Hahn J, Voth M (1994) Dynamics of mitochondria in living cells: shape changes, dislocations, fusion, and fission of mitochondria. *Microsc Res Tech* 27:198-219.
- Bertholet AM, Millet AM, Guillermin O, Daloyau M, Davezac N, Miquel MC, Belenguer P (2013) OPA1 loss of function affects in vitro neuronal maturation. *Brain* 136:1518-1533.
- Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT (2000) Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci* 3:1301-1306.
- Beyer K, Nuscher B (1996) Specific cardiolipin binding interferes with labeling of sulfhydryl residues in the adenosine diphosphate/adenosine triphosphate carrier protein from beef heart mitochondria. *Biochemistry* 35:15784-15790.
- Billups B, Forsythe ID (2002) Presynaptic mitochondrial calcium sequestration influences transmission at mammalian central synapses. *J Neurosci* 22:5840-5847.
- Bilsland LG, Sahai E, Kelly G, Golding M, Greensmith L, Schiavo G (2010) Deficits in axonal transport precede ALS symptoms in vivo. *Proc Natl Acad Sci U S A* 107:20523-20528.
- Bitto H, Deisseroth K, Tsien RW (1996) CREB phosphorylation and dephosphorylation: a Ca(2+)- and stimulus duration-dependent switch for hippocampal gene expression. *Cell* 87:1203-1214.

- Boland B, Kumar A, Lee S, Platt FM, Wegiel J, Yu WH, Nixon RA (2008) Autophagy induction and autophagosome clearance in neurons: relationship to autophagic pathology in Alzheimer's disease. *J Neurosci* 28:6926-6937.
- Bolanos JP, Almeida A, Moncada S (2010) Glycolysis: a bioenergetic or a survival pathway? *Trends Biochem Sci* 35:145-149.
- Bossy B, Petrilli A, Klinglmayr E, Chen J, Lutz-Meindl U, Knott AB, Masliah E, Schwarzenbacher R, Bossy-Wetzel E (2010) S-Nitrosylation of DRP1 does not affect enzymatic activity and is not specific to Alzheimer's disease. *J Alzheimers Dis* 20 Suppl 2:S513-S526.
- Bossy-Wetzel E, Petrilli A, Knott AB (2008) Mutant huntingtin and mitochondrial dysfunction. *Trends Neurosci* 31:609-616.
- Bossy-Wetzel E, Schwarzenbacher R, Lipton SA (2004) Molecular pathways to neurodegeneration. *Nat Med* 10 Suppl:S2-S9.
- Braak H, Del TK, Rub U, de Vos RA, Jansen Steur EN, Braak E (2003) Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging* 24:197-211.
- Braak H, Ghebremedhin E, Rub U, Bratzke H, Del TK (2004) Stages in the development of Parkinson's disease-related pathology. *Cell Tissue Res* 318:121-134.
- Braschi E, Zunino R, McBride HM (2009) MAPL is a new mitochondrial SUMO E3 ligase that regulates mitochondrial fission. *EMBO Rep* 10:748-754.
- Caccamo A, Majumder S, Richardson A, Strong R, Oddo S (2010) Molecular interplay between mammalian target of rapamycin (mTOR), amyloid-beta, and Tau: effects on cognitive impairments. *J Biol Chem* 285:13107-13120.
- Cai Q, Gerwin C, Sheng ZH (2005) Syntabulin-mediated anterograde transport of mitochondria along neuronal processes. *J Cell Biol* 170:959-969.
- Cai Q, Zakaria HM, Simone A, Sheng ZH (2012) Spatial parkin translocation and degradation of damaged mitochondria via mitophagy in live cortical neurons. *Curr Biol* 22:545-552.
- Campello S, Scorrano L (2010) Mitochondrial shape changes: orchestrating cell pathophysiology. *EMBO Rep* 11:678-684.
- Canto C, Auwerx J (2010) AMP-activated protein kinase and its downstream transcriptional pathways. *Cell Mol Life Sci* 67:3407-3423.
- Cardenas C, Miller RA, Smith I, Bui T, Molgo J, Muller M, Vais H, Cheung KH, Yang J, Parker I, Thompson CB, Birnbaum MJ, Hallows KR, Fosskett JK (2010) Essential regulation of cell bioenergetics by constitutive InsP3 receptor Ca2+ transfer to mitochondria. *Cell* 142:270-283.
- Carelli V, Ross-Cisneros FN, Sadun AA (2002) Optic nerve degeneration and mitochondrial dysfunction: genetic and acquired optic neuropathies. *Neurochem Int* 40:573-584.
- Carelli V, Ross-Cisneros FN, Sadun AA (2004) Mitochondrial dysfunction as a cause of optic neuropathies. *Prog Retin Eye Res* 23:53-89.
- Cereghetti GM, Stangherlin A, Martins de BO, Chang CR, Blackstone C, Bernardi P, Scorrano L (2008a) Dephosphorylation by calcineurin regulates translocation of Drp1 to mitochondria. *Proc Natl Acad Sci U S A* 105:15803-15808.
- Cereghetti GM, Stangherlin A, Martins de BO, Chang CR, Blackstone C, Bernardi P, Scorrano L (2008b) Dephosphorylation by calcineurin regulates translocation of Drp1 to mitochondria. *Proc Natl Acad Sci U S A* 105:15803-15808.
- Chada SR, Hollenbeck PJ (2004) Nerve growth factor signaling regulates motility and docking of axonal mitochondria. *Curr Biol* 14:1272-1276.
- Chan NC, Salazar AM, Pham AH, Sweredoski MJ, Kolawa NJ, Graham RL, Hess S, Chan DC (2011) Broad activation of the ubiquitin-proteasome system by Parkin is critical for mitophagy. *Hum Mol Genet* 20:1726-1737.
- Chang DT, Rintoul GL, Pandipati S, Reynolds IJ (2006) Mutant huntingtin aggregates impair mitochondrial movement and trafficking in cortical neurons. *Neurobiol Dis* 22:388-400.
- Chang KT, Niescier RF, Min KT (2011) Mitochondrial matrix Ca2+ as an intrinsic signal regulating mitochondrial motility in axons. *Proc Natl Acad Sci U S A* 108:15456-15461.
- Chen H, Chomyn A, Chan DC (2005) Disruption of fusion results in mitochondrial heterogeneity and dysfunction. *J Biol Chem* 280:26185-26192.
- Chen H, Detmer SA, Ewald AJ, Griffin EE, Fraser SE, Chan DC (2003a) Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. *J Cell Biol* 160:189-200.

Chen H, Detmer SA, Ewald AJ, Griffin EE, Fraser SE, Chan DC (2003b) Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. *J Cell Biol* 160:189-200.

Chen H, Vermulst M, Wang YE, Chomyn A, Prolla TA, McCaffery JM, Chan DC (2010) Mitochondrial fusion is required for mtDNA stability in skeletal muscle and tolerance of mtDNA mutations. *Cell* 141:280-289.

Chen S, Owens GC, Crossin KL, Edelman DB (2007) Serotonin stimulates mitochondrial transport in hippocampal neurons. *Mol Cell Neurosci* 36:472-483.

Chen S, Owens GC, Edelman DB (2008) Dopamine inhibits mitochondrial motility in hippocampal neurons. *PLoS One* 3:e2804.

Chen YM, Gerwin C, Sheng ZH (2009) Dynein light chain LC8 regulates syntaphilin-mediated mitochondrial docking in axons. *J Neurosci* 29:9429-9438.

Cheung ZH, Ip NY (2009) The emerging role of autophagy in Parkinson's disease. *Mol Brain* 2:29.

Chinta SJ, Mallajosyula JK, Rane A, Andersen JK (2010) Mitochondrial alpha-synuclein accumulation impairs complex I function in dopaminergic neurons and results in increased mitophagy in vivo. *Neurosci Lett* 486:235-239.

Cho DH, Nakamura T, Fang J, Cieplak P, Godzik A, Gu Z, Lipton SA (2009) S-nitrosylation of Drp1 mediates beta-amyloid-related mitochondrial fission and neuronal injury. *Science* 324:102-105.

Cho KI, Cai Y, Yi H, Yeh A, Aslanukov A, Ferreira PA (2007) Association of the kinesin-binding domain of RanBP2 to KIF5B and KIF5C determines mitochondria localization and function. *Traffic* 8:1722-1735.

Choi SY, Huang P, Jenkins GM, Chan DC, Schiller J, Frohman MA (2006) A common lipid links Mfn-mediated mitochondrial fusion and SNARE-regulated exocytosis. *Nat Cell Biol* 8:1255-1262.

Choi YJ, Di NA, Kramvis I, Meikle L, Kwiatkowski DJ, Sahin M, He X (2008) Tuberous sclerosis complex proteins control axon formation. *Genes Dev* 22:2485-2495.

Chu CT, et al. (2013) Cardiolipin externalization to the outer mitochondrial membrane acts as an elimination signal for mitophagy in neuronal cells. *Nat Cell Biol* 15:1197-1205.

Cipolat S, de Brito OM, Dal Zilio B, Scorrano L (2004a) OPA(1) requires mitofusin-1 to promote mitochondrial fusion. pp 38.

Cipolat S, Martins de BO, Dal ZB, Scorrano L (2004b) OPA1 requires mitofusin 1 to promote mitochondrial fusion. *Proc Natl Acad Sci U S A* 101:15927-15932.

Cipolat S, Rudka T, Hartmann D, Costa V, Serneels L, Craessaerts K, Metzger K, Frezza C, Annaert W, D'Adamio L, Derks C, Dejaegere T, Pellegrini L, D'Hooge R, Scorrano L, De Strooper B (2006a) Mitochondrial Rhomboid PARL Regulates Cytochrome c Release during Apoptosis via OPA1-Dependent Cristae Remodeling. *Cell* 126:163-175.

Cipolat S, Rudka T, Hartmann D, Costa V, Serneels L, Craessaerts K, Metzger K, Frezza C, Annaert W, D'Adamio L, Derks C, Dejaegere T, Pellegrini L, D'Hooge R, Scorrano L, De SB (2006b) Mitochondrial rhomboid PARL regulates cytochrome c release during apoptosis via OPA1-dependent cristae remodeling. *Cell* 126:163-175.

Clark LN, Wang Y, Karlins E, Saito L, Mejia-Santana H, Harris J, Louis ED, Cote LJ, Andrews H, Fahn S, Waters C, Ford B, Frucht S, Ottman R, Marder K (2006) Frequency of LRRK2 mutations in early- and late-onset Parkinson disease. *Neurology* 67:1786-1791.

Claude A (1946) FRACTIONATION OF MAMMALIAN LIVER CELLS BY DIFFERENTIAL CENTRIFUGATION : II. EXPERIMENTAL PROCEDURES AND RESULTS. *J Exp Med* 84:61-89.

Cogliati S, Frezza C, Soriano ME, Varanita T, Quintana-Cabrera R, Corrado M, Cipolat S, Costa V, Casarin A, Gomes LC, Perales-Clemente E, Salviati L, Fernandez-Silva P, Enriquez JA, Scorrano L (2013) Mitochondrial cristae shape determines respiratory chain supercomplexes assembly and respiratory efficiency. *Cell* 155:160-171.

Cook JE, Chalupa LM (2000) Retinal mosaics: new insights into an old concept. *Trends Neurosci* 23:26-34.

Costa V, Giacomello M, Hudec R, Lopreiato R, Ermak G, Lim D, Malorni W, Davies KJ, Carafoli E, Scorrano L (2010) Mitochondrial fission and cristae disruption increase the response of cell models of Huntington's disease to apoptotic stimuli. *EMBO Mol Med* 2:490-503.

Cozzolino M, Pesaresi MG, Amori I, Crosio C, Ferri A, Nencini M, Carri MT (2009) Oligomerization of mutant SOD1 in mitochondria of motoneuronal cells drives mitochondrial damage and cell toxicity. *Antioxid Redox Signal* 11:1547-1558.

Cui L, Jeong H, Borovecki F, Parkhurst CN, Tanese N, Krainc D (2006) Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. *Cell* 127:59-69.

- Culmsee C, Monnig J, Kemp BE, Mattson MP (2001) AMP-activated protein kinase is highly expressed in neurons in the developing rat brain and promotes neuronal survival following glucose deprivation. *J Mol Neurosci* 17:45-58.
- Cummings JL (2009) Defining and labeling disease-modifying treatments for Alzheimer's disease. *Alzheimers Dement* 5:406-418.
- Dauer W, Przedborski S (2003) Parkinson's disease: mechanisms and models. *Neuron* 39:889-909.
- Davies VJ, Hollins AJ, Piechota MJ, Yip W, Davies JR, White KE, Nicols PP, Boulton ME, Votruba M (2007) Opa1 deficiency in a mouse model of autosomal dominant optic atrophy impairs mitochondrial morphology, optic nerve structure and visual function. *Hum Mol Genet* 16:1307-1318.
- Dayanithi G, Chen-Kuo-Chang M, Viero C, Hamel C, Muller A, Lenaers G (2010) Characterization of Ca²⁺ signalling in postnatal mouse retinal ganglion cells: involvement of OPA1 in Ca²⁺ clearance. *Ophthalmic Genet* 31:53-65.
- de Brito OM, Scorrano L (2008a) Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature* 456:605-610.
- de Brito OM, Scorrano L (2008b) Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature* 456:605-610.
- De Vos KJ, Chapman AL, Tennant ME, Manser C, Tudor EL, Lau KF, Brownlees J, Ackerley S, Shaw PJ, McLoughlin DM, Shaw CE, Leigh PN, Miller CC, Grierson AJ (2007) Familial amyotrophic lateral sclerosis-linked SOD1 mutants perturb fast axonal transport to reduce axonal mitochondria content. *Hum Mol Genet* 16:2720-2728.
- De SD, Raffaello A, Teardo E, Szabo I, Rizzuto R (2011) A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter. *Nature* 476:336-340.
- Deas E, Plun-Favreau H, Gandhi S, Desmond H, Kjaer S, Loh SH, Renton AE, Harvey RJ, Whitworth AJ, Martins LM, Abramov AY, Wood NW (2011) PINK1 cleavage at position A103 by the mitochondrial protease PARL. *Hum Mol Genet* 20:867-879.
- Delettre C, Griffoin JM, Kaplan J, Dollfus H, Lorenz B, Faivre L, Lenaers G, Belenguer P, Hamel CP (2001) Mutation spectrum and splicing variants in the OPA1 gene. *Hum Genet* 109:584-591.
- Delettre C, Lenaers G, Griffoin JM, Gigarel N, Lorenzo C, Belenguer P, Pelloquin L, Grosgeorge J, Turc-Carel C, Perret E, Astarie-Dequeker C, Lasquelléc L, Arnaud B, Ducommun B, Kaplan J, Hamel CP (2000a) Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. *Nat Genet* 26:207-210.
- Delettre C, Lenaers G, Griffoin JM, Gigarel N, Lorenzo C, Belenguer P, Pelloquin L, Grosgeorge J, Turc-Carel C, Perret E, Astarie-Dequeker C, Lasquelléc L, Arnaud B, Ducommun B, Kaplan J, Hamel CP (2000b) Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. *Nat Genet* 26:207-210.
- Demongeot J, Glade N, Hansen O, Moreira A (2007) An open issue: the inner mitochondrial membrane (IMM) as a free boundary problem. *Biochimie* 89:1049-1057.
- Deng H, Dodson MW, Huang H, Guo M (2008) The Parkinson's disease genes pink1 and parkin promote mitochondrial fission and/or inhibit fusion in *Drosophila*. *Proc Natl Acad Sci U S A* 105:14503-14508.
- Devi L, Prabhu BM, Galati DF, Avadhani NG, Anandatheerthavarada HK (2006) Accumulation of amyloid precursor protein in the mitochondrial import channels of human Alzheimer's disease brain is associated with mitochondrial dysfunction. *J Neurosci* 26:9057-9068.
- Devi L, Raghavendran V, Prabhu BM, Avadhani NG, Anandatheerthavarada HK (2008) Mitochondrial import and accumulation of alpha-synuclein impair complex I in human dopaminergic neuronal cultures and Parkinson disease brain. *J Biol Chem* 283:9089-9100.
- Dimmer KS, Navoni F, Casarin A, Trevisson E, Endeles S, Winterpacht A, Salviati L, Scorrano L (2008) LETM1, deleted in Wolf Hirschhorn syndrome is required for normal mitochondrial morphology and cellular viability. *Hum Mol Genet* 17:201-214.
- Dixit R, Ross JL, Goldman YE, Holzbaur EL (2008) Differential regulation of dynein and kinesin motor proteins by tau. *Science* 319:1086-1089.
- Duffy LM, Chapman AL, Shaw PJ, Grierson AJ (2011) Review: The role of mitochondria in the pathogenesis of amyotrophic lateral sclerosis. *Neuropathol Appl Neurobiol* 37:336-352.
- Duvezin-Caubet S, Koppen M, Wagener J, Zick M, Israel L, Bernacchia A, Jagasia R, Rugarli EI, Imhof A, Neupert W, Langer T, Reichert AS (2007) OPA1 processing reconstituted in yeast depends on the subunit composition of the m-AAA protease in mitochondria. *Mol Biol Cell* 18:3582-3590.
- Ehres S, Raschke I, Mancuso G, Bernacchia A, Geimer S, Tondera D, Martinou JC, Westermann B, Rugarli EI, Langer T (2009) Regulation of OPA1 processing and mitochondrial fusion by m-AAA protease isoenzymes and OMA1. *J Cell Biol* 187:1023-1036.
- Eskelinen EL (2005) Maturation of autophagic vacuoles in Mammalian cells. *Autophagy* 1:1-10.

- Estela A, Pla-Martin D, Sanchez-Piris M, Sesaki H, Palau F (2011) Charcot-Marie-Tooth-related gene GDAP1 complements cell cycle delay at G2/M phase in *Saccharomyces cerevisiae* fis1 gene-defective cells. *J Biol Chem* 286:36777-36786.
- Eura Y, Ishihara N, Oka T, Mihara K (2006) Identification of a novel protein that regulates mitochondrial fusion by modulating mitofusin (Mfn) protein function. *J Cell Sci* 119:4913-4925.
- Eura Y, Ishihara N, Yokota S, Mihara K (2003) Two mitofusin proteins, mammalian homologues of FZO, with distinct functions are both required for mitochondrial fusion. *J Biochem (Tokyo)* 134:333-344.
- Famiglietti EV, Jr., Kolb H (1976) Structural basis for ON-and OFF-center responses in retinal ganglion cells. *Science* 194:193-195.
- Ferre M, Amati-Bonneau P, Tourmen Y, Malthiery Y, Reynier P (2005) eOPA1: an online database for OPA1 mutations. *Hum Mutat* 25:423-428.
- Ferri A, Fiorenzo P, Nencini M, Cozzolino M, Pesaresi MG, Valle C, Sepe S, Moreno S, Carri MT (2010) Glutaredoxin 2 prevents aggregation of mutant SOD1 in mitochondria and abolishes its toxicity. *Hum Mol Genet* 19:4529-4542.
- Fimia GM, Stoykova A, Romagnoli A, Giunta L, Di BS, Nardacci R, Corazzari M, Fuoco C, Ucar A, Schwartz P, Gruss P, Piacentini M, Chowdhury K, Cecconi F (2007) Ambra1 regulates autophagy and development of the nervous system. *Nature* 447:1121-1125.
- Fischer LR, Igoudjil A, Magrane J, Li Y, Hansen JM, Manfredi G, Glass JD (2011) SOD1 targeted to the mitochondrial intermembrane space prevents motor neuropathy in the Sod1 knockout mouse. *Brain* 134:196-209.
- Fransson S, Ruusala A, Aspenstrom P (2006) The atypical Rho GTPases Miro-1 and Miro-2 have essential roles in mitochondrial trafficking. *Biochem Biophys Res Commun* 344:500-510.
- Frederick RL, McCaffery JM, Cunningham KW, Okamoto K, Shaw JM (2004) Yeast Miro GTPase, Gem1p, regulates mitochondrial morphology via a novel pathway. *J Cell Biol* 167:87-98.
- Frederick RL, Shaw JM (2007) Moving mitochondria: establishing distribution of an essential organelle. *Traffic* 8:1668-1675.
- Frey TG, Mannella CA (2000) The internal structure of mitochondria. *Trends Biochem Sci* 25:319-324.
- Frezza C, Cipolat S, Martins de BO, Micaroni M, Beznoussenko GV, Rudka T, Bartoli D, Polishuck RS, Danial NN, De SB, Scorrano L (2006a) OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion. *Cell* 126:177-189.
- Frezza C, Cipolat S, Martins dB, Micaroni M, Beznoussenko GV, Rudka T, Bartoli D, Polishuck RS, Danial NN, De Strooper B, Scorrano L (2006b) OPA1 Controls Apoptotic Cristae Remodeling Independently from Mitochondrial Fusion. *Cell* 126:177-189.
- Friedman LG, Lachenmayer ML, Wang J, He L, Poulouse SM, Komatsu M, Holstein GR, Yue Z (2012) Disrupted autophagy leads to dopaminergic axon and dendrite degeneration and promotes presynaptic accumulation of alpha-synuclein and LRRK2 in the brain. *J Neurosci* 32:7585-7593.
- Fujita T, Maturana AD, Ikuta J, Hamada J, Walchli S, Suzuki T, Sawa H, Wooten MW, Okajima T, Tatematsu K, Tanizawa K, Kuroda S (2007) Axonal guidance protein FEZ1 associates with tubulin and kinesin motor protein to transport mitochondria in neurites of NGF-stimulated PC12 cells. *Biochem Biophys Res Commun* 361:605-610.
- Furuta N, Yoshimori T, Amano A (2010) Mediator molecules that fuse autophagosomes and lysosomes. *Autophagy* 6:417-418.
- Galli-Resta L, Leone P, Bottari D, Ensini M, Rigosi E, Novelli E (2008) The genesis of retinal architecture: an emerging role for mechanical interactions? *Prog Retin Eye Res* 27:260-283.
- Garraghty PE, Sur M (1993) Competitive interactions influencing the development of retinal axonal arbors in cat lateral geniculate nucleus. *Physiol Rev* 73:529-545.
- Gegg ME, Cooper JM, Chau KY, Rojo M, Schapira AH, Taanman JW (2010) Mitofusin 1 and mitofusin 2 are ubiquitinated in a PINK1/parkin-dependent manner upon induction of mitophagy. *Hum Mol Genet* 19:4861-4870.
- Geisler S, Holmstrom KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, Springer W (2010) PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. *Nat Cell Biol* 12:119-131.
- Gilkerson RW, Selker JM, Capaldi RA (2003) The cristal membrane of mitochondria is the principal site of oxidative phosphorylation. *FEBS Lett* 546:355-358.
- Giordano C, Montopoli M, Perli E, Orlandi M, Fantin M, Ross-Cisneros FN, Caparrotta L, Martinuzzi A, Ragazzi E, Ghelli A, Sadun AA, d'Amati G, Carelli V (2011) Oestrogens ameliorate mitochondrial dysfunction in Leber's hereditary optic neuropathy. *Brain* 134:220-234.

Giraud MF, Paumard P, Soubannier V, Vaillier J, Arselin G, Salin B, Schaeffer J, Brethes D, di Rago JP, Velours J (2002) Is there a relationship between the supramolecular organization of the mitochondrial ATP synthase and the formation of cristae? *Biochim Biophys Acta* 1555:174-180.

Glater EE, Megeath LJ, Stowers RS, Schwarz TL (2006) Axonal transport of mitochondria requires milton to recruit kinesin heavy chain and is light chain independent. *J Cell Biol* 173:545-557.

Goorden SM, van Woerden GM, van der Weerd L, Cheadle JP, Elgersma Y (2007) Cognitive deficits in Tsc1^{+/-} mice in the absence of cerebral lesions and seizures. *Ann Neurol* 62:648-655.

Greene AW, Grenier K, Aguilera MA, Muise S, Farazifard R, Haque ME, McBride HM, Park DS, Fon EA (2012) Mitochondrial processing peptidase regulates PINK1 processing, import and Parkin recruitment. *EMBO Rep* 13:378-385.

Greene JC, Whitworth AJ, Kuo I, Andrews LA, Feany MB, Pallanck LJ (2003) Mitochondrial pathology and apoptotic muscle degeneration in *Drosophila* parkin mutants. *Proc Natl Acad Sci U S A* 100:4078-4083.

Grenier K, McLelland GL, Fon EA (2013) Parkin- and PINK1-Dependent Mitophagy in Neurons: Will the Real Pathway Please Stand Up? *Front Neurol* 4:100.

Griparic L, van der Wel NN, Orozco IJ, Peters PJ, van der Blik AM (2004a) Loss of the intermembrane space protein Mgm1/OPA1 induces swelling and localized constrictions along the lengths of mitochondria. *J Biol Chem* 279:18792-18798.

Griparic L, van der Wel NN, Orozco IJ, Peters PJ, van der Blik AM (2004b) Loss of the intermembrane space protein Mgm1/OPA1 induces swelling and localized constrictions along the lengths of mitochondria. *J Biol Chem* 279:18792-18798.

Grishin A, Li H, Levitan ES, Zaks-Makhina E (2006) Identification of gamma-aminobutyric acid receptor-interacting factor 1 (TRAK2) as a trafficking factor for the K⁺ channel Kir2.1. *J Biol Chem* 281:30104-30111.

Groth RD, Dunbar RL, Mermelstein PG (2003) Calcineurin regulation of neuronal plasticity. *Biochem Biophys Res Commun* 311:1159-1171.

Guillery O, Malka F, Landes T, Guillou E, Blackstone C, Lombes A, Belenguer P, Arnoult D, Rojo M (2008) Metalloprotease-mediated OPA1 processing is modulated by the mitochondrial membrane potential. *Biol Cell* 100:315-325.

Guillou E, Bousquet C, Daloyau M, Emorine LJ, Belenguer P (2005) Msp1p is an intermembrane space dynamin-related protein that mediates mitochondrial fusion in a Dnm1p-dependent manner in *S. pombe*. *FEBS Lett* 579:1109-1116.

Guo X, Macleod GT, Wellington A, Hu F, Panchumarthi S, Schoenfield M, Marin L, Charlton MP, Atwood HL, Zinsmaier KE (2005) The GTPase dMiro is required for axonal transport of mitochondria to *Drosophila* synapses. *Neuron* 47:379-393.

Hackenbrock CR (1968) Chemical and physical fixation of isolated mitochondria in low-energy and high-energy states. *Proc Natl Acad Sci U S A* 61:598-605.

Hackenbrock CR, Schneider H, Lemasters JJ, Hochli M (1980) Relationships between bilayer lipid, motional freedom of oxidoreductase components, and electron transfer in the mitochondrial inner membrane. *Adv Exp Med Biol* 132:245-263.

Hailey DW, Rambold AS, Satpute-Krishnan P, Mitra K, Sougrat R, Kim PK, Lippincott-Schwartz J (2010) Mitochondria supply membranes for autophagosome biogenesis during starvation. *Cell* 141:656-667.

Hales KG, Fuller MT (1997) Developmentally regulated mitochondrial fusion mediated by a conserved, novel, predicted GTPase. *Cell* 90:121-129.

Han XJ, Lu YF, Li SA, Kaitsuka T, Sato Y, Tomizawa K, Nairn AC, Takei K, Matsui H, Matsushita M (2008a) CaM kinase I alpha-induced phosphorylation of Drp1 regulates mitochondrial morphology. *J Cell Biol* 182:573-585.

Han XJ, Lu YF, Li SA, Tomizawa K, Takei K, Matsushita M, Matsui H (2008b) Involvement of calcineurin in glutamate-induced mitochondrial dynamics in neurons. *Neurosci Res* 60:114-119.

Haque ME, Mount MP, Safarpour F, Abdel-Messih E, Callaghan S, Mazerolle C, Kitada T, Slack RS, Wallace V, Shen J, Anisman H, Park DS (2012) Inactivation of Pink1 gene in vivo sensitizes dopamine-producing neurons to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and can be rescued by autosomal recessive Parkinson disease genes, Parkin or DJ-1. *J Biol Chem* 287:23162-23170.

Hardie DG (2008) AMPK: a key regulator of energy balance in the single cell and the whole organism. *Int J Obes (Lond)* 32 Suppl 4:S7-12.

Hayashi-Nishino M, Fujita N, Noda T, Yamaguchi A, Yoshimori T, Yamamoto A (2009) A subdomain of the endoplasmic reticulum forms a cradle for autophagosome formation. *Nat Cell Biol* 11:1433-1437.

- Head B, Griparic L, Amiri M, Gandre-Babbe S, van der Blik AM (2009) Inducible proteolytic inactivation of OPA1 mediated by the OMA1 protease in mammalian cells. *J Cell Biol* 187:959-966.
- Herlan M, Vogel F, Bornhovd C, Neupert W, Reichert AS (2003) Processing of Mgm1 by the rhomboid-type protease Pcp1 is required for maintenance of mitochondrial morphology and of mitochondrial DNA. *J Biol Chem* 278:27781-27788.
- Hermann GJ, Thatcher JW, Mills JP, Hales KG, Fuller MT, Nunnari J, Shaw JM (1998) Mitochondrial fusion in yeast requires the transmembrane GTPase Fzo1p. *J Cell Biol* 143:359-373.
- Hinshaw JE (1999) Dynamin spirals. *Curr Opin Struct Biol* 9:260-267.
- Hogan V, White K, Edgar J, McGill A, Karim S, McLaughlin M, Griffiths I, Turnbull D, Nichols P (2009) Increase in mitochondrial density within axons and supporting cells in response to demyelination in the Plp1 mouse model. *J Neurosci Res* 87:452-459.
- Hong S, Iizuka Y, Kim CY, Seong GJ (2012) Isolation of primary mouse retinal ganglion cells using immunopanning-magnetic separation. *Mol Vis* 18:2922-2930.
- Hoshino A, Mita Y, Okawa Y, Ariyoshi M, Iwai-Kanai E, Ueyama T, Ikeda K, Ogata T, Matoba S (2013) Cytosolic p53 inhibits Parkin-mediated mitophagy and promotes mitochondrial dysfunction in the mouse heart. *Nat Commun* 4:2308.
- Ikuta J, Maturana A, Fujita T, Okajima T, Tatematsu K, Tanizawa K, Kuroda S (2007) Fasciculation and elongation protein zeta-1 (FEZ1) participates in the polarization of hippocampal neuron by controlling the mitochondrial motility. *Biochem Biophys Res Commun* 353:127-132.
- Irrcher I, et al. (2010) Loss of the Parkinson's disease-linked gene DJ-1 perturbs mitochondrial dynamics. *Hum Mol Genet* 19:3734-3746.
- Irwin WA, Bergamin N, Sabatelli P, Reggiani C, Megighian A, Merlini L, Braghetta P, Columbaro M, Volpin D, Bressan GM, Bernardi P, Bonaldo P (2003) Mitochondrial dysfunction and apoptosis in myopathic mice with collagen VI deficiency. *Nat Genet* 35:367-371.
- Ishihara N, Eura Y, Mihara K (2004) Mitofusin 1 and 2 play distinct roles in mitochondrial fusion reactions via GTPase activity. *J Cell Sci* 117:6535-6546.
- Ishihara N, Fujita Y, Oka T, Mihara K (2006) Regulation of mitochondrial morphology through proteolytic cleavage of OPA1. *EMBO J* 25:2966-2977.
- Israelson A, Arbel N, Da CS, Ilieva H, Yamanaka K, Shoshan-Barmatz V, Cleveland DW (2010) Misfolded mutant SOD1 directly inhibits VDAC1 conductance in a mouse model of inherited ALS. *Neuron* 67:575-587.
- Itoh K, Nakamura K, Iijima M, Sesaki H (2013) Mitochondrial dynamics in neurodegeneration. *Trends Cell Biol* 23:64-71.
- Ivanova E, Hwang GS, Pan ZH (2010) Characterization of transgenic mouse lines expressing Cre recombinase in the retina. *Neuroscience* 165:233-243.
- Jahani-Asl A, Pilon-Larose K, Xu W, MacLaurin JG, Park DS, McBride HM, Slack RS (2011) The mitochondrial inner membrane GTPase, optic atrophy 1 (Opa1), restores mitochondrial morphology and promotes neuronal survival following excitotoxicity. *J Biol Chem* 286:4772-4782.
- James DI, Parone PA, Mattenberger Y, Martinou JC (2003) hFis1, a novel component of the mammalian mitochondrial fission machinery. *J Biol Chem* 278:36373-36379.
- Jansen M, Ten Klooster JP, Offerhaus GJ, Clevers H (2009) LKB1 and AMPK family signaling: the intimate link between cell polarity and energy metabolism. *Physiol Rev* 89:777-798.
- Jellinger KA (2012) Neuropathology of sporadic Parkinson's disease: evaluation and changes of concepts. *Mov Disord* 27:8-30.
- Jiang H, Guo W, Liang X, Rao Y (2005) Both the establishment and the maintenance of neuronal polarity require active mechanisms: critical roles of GSK-3beta and its upstream regulators. *Cell* 120:123-135.
- Jimenez-Mateos EM, Gonzalez-Billault C, Dawson HN, Vitek MP, Avila J (2006) Role of MAP1B in axonal retrograde transport of mitochondria. *Biochem J* 397:53-59.
- Johnston PB, Gaster RN, Smith VC, Tripathi RC (1979) A clinicopathologic study of autosomal dominant optic atrophy. *Am J Ophthalmol* 88:868-875.
- Jones BA, Fangman WL (1992) Mitochondrial DNA maintenance in yeast requires a protein containing a region related to the GTP-binding domain of dynamin. *Genes Dev* 6:380-389.

- Joselin AP, Hewitt SJ, Callaghan SM, Kim RH, Chung YH, Mak TW, Shen J, Slack RS, Park DS (2012) ROS-dependent regulation of Parkin and DJ-1 localization during oxidative stress in neurons. *Hum Mol Genet* 21:4888-4903.
- Kamei S, Chen-Kuo-Chang M, Cazevielle C, Lenaers G, Olichon A, Belenguer P, Roussignol G, Renard N, Eybalin M, Michelin A, Delettre C, Brabet P, Hamel CP (2005) Expression of the Opa1 mitochondrial protein in retinal ganglion cells: its downregulation causes aggregation of the mitochondrial network. *Invest Ophthalmol Vis Sci* 46:4288-4294.
- Kamp F, Exner N, Lutz AK, Wender N, Hegermann J, Brunner B, Nuscher B, Bartels T, Giese A, Beyer K, Eimer S, Winklhofer KF, Haass C (2010) Inhibition of mitochondrial fusion by alpha-synuclein is rescued by PINK1, Parkin and DJ-1. *EMBO J* 29:3571-3589.
- Kanai Y, Okada Y, Tanaka Y, Harada A, Terada S, Hirokawa N (2000) KIF5C, a novel neuronal kinesin enriched in motor neurons. *J Neurosci* 20:6374-6384.
- Kang JS, Tian JH, Pan PY, Zald P, Li C, Deng C, Sheng ZH (2008) Docking of axonal mitochondria by syntaphilin controls their mobility and affects short-term facilitation. *Cell* 132:137-148.
- Karbowski M, Neutzner A, Youle RJ (2007) The mitochondrial E3 ubiquitin ligase MARCH5 is required for Drp1 dependent mitochondrial division. *J Cell Biol* 178:71-84.
- Karbowski M, Norris KL, Cleland MM, Jeong SY, Youle RJ (2006) Role of Bax and Bak in mitochondrial morphogenesis. *Nature* 443:658-662.
- Kennedy EP, Lehninger AL (1949) Oxidation of fatty acids and tricarboxylic acid cycle intermediates by isolated rat liver mitochondria. *J Biol Chem* 179:957-972.
- Khandelwal PJ, Herman AM, Hoe HS, Rebeck GW, Moussa CE (2011) Parkin mediates beclin-dependent autophagic clearance of defective mitochondria and ubiquitinated Abeta in AD models. *Hum Mol Genet* 20:2091-2102.
- Kijima K, Numakura C, Izumino H, Umetsu K, Nezu A, Shiiki T, Ogawa M, Ishizaki Y, Kitamura T, Shozawa Y, Hayasaka K (2005) Mitochondrial GTPase mitofusin 2 mutation in Charcot-Marie-Tooth neuropathy type 2A. *Hum Genet* 116:23-27.
- Kim J, Kundu M, Viollet B, Guan KL (2011) AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol* 13:132-141.
- Kincaid RL, Balaban CD, Billingsley ML (1986) Regulated expression of calmodulin-dependent cyclic nucleotide phosphodiesterase in the central nervous system. *J Cyclic Nucleotide Protein Phosphor Res* 11:473-486.
- Kirisako T, Ichimura Y, Okada H, Kabeya Y, Mizushima N, Yoshimori T, Ohsumi M, Takao T, Noda T, Ohsumi Y (2000) The reversible modification regulates the membrane-binding state of Apg8/Aut7 essential for autophagy and the cytoplasm to vacuole targeting pathway. *J Cell Biol* 151:263-276.
- Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N (1998) Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 392:605-608.
- Kjer P, Jensen OA, Klinken L (1983) Histopathology of eye, optic nerve and brain in a case of dominant optic atrophy. *Acta Ophthalmol (Copenh)* 61:300-312.
- Kocsis E, Trus BL, Steer CJ, Bisher ME, Steven AC (1991) Image averaging of flexible fibrous macromolecules: the clathrin triskelion has an elastic proximal segment. *J Struct Biol* 107:6-14.
- Komatsu M, Wang QJ, Holstein GR, Friedrich VL, Jr., Iwata J, Kominami E, Chait BT, Tanaka K, Yue Z (2007) Essential role for autophagy protein Atg7 in the maintenance of axonal homeostasis and the prevention of axonal degeneration. *Proc Natl Acad Sci U S A* 104:14489-14494.
- Korolchuk VI, Menzies FM, Rubinsztein DC (2010) Mechanisms of cross-talk between the ubiquitin-proteasome and autophagy-lysosome systems. *FEBS Lett* 584:1393-1398.
- Koshiba T, Detmer SA, Kaiser JT, Chen H, McCaffery JM, Chan DC (2004) Structural basis of mitochondrial tethering by mitofusin complexes. *Science* 305:858-862.
- Kushnareva YE, Gerencser AA, Bossy B, Ju WK, White AD, Waggoner J, Ellisman MH, Perkins G, Bossy-Wetzel E (2013) Loss of OPA1 disturbs cellular calcium homeostasis and sensitizes for excitotoxicity. *Cell Death Differ* 20:353-365.
- Labrousse AM, Zappaterra MD, Rube DA, van der Bliek AM (1999) C. elegans dynamin-related protein DRP-1 controls severing of the mitochondrial outer membrane. *Mol Cell* 4:815-826.
- Laird FM, Farah MH, Ackerley S, Hoke A, Maragakis N, Rothstein JD, Griffin J, Price DL, Martin LJ, Wong PC (2008) Motor neuron disease occurring in a mutant dynactin mouse model is characterized by defects in vesicular trafficking. *J Neurosci* 28:1997-2005.

- Lawson VH, Graham BV, Flanigan KM (2005) Clinical and electrophysiologic features of CMT2A with mutations in the mitofusin 2 gene. *Neurology* 65:197-204.
- Lazarou M, Jin SM, Kane LA, Youle RJ (2012) Role of PINK1 binding to the TOM complex and alternate intracellular membranes in recruitment and activation of the E3 ligase Parkin. *Dev Cell* 22:320-333.
- Lechaue C, Augustin S, Cwerman-Thibault H, Bouaita A, Forster V, Celier C, Rustin P, Marden MC, Sahel JA, Corral-Debrinski M (2012) Neuroglobin involvement in respiratory chain function and retinal ganglion cell integrity. *Biochim Biophys Acta* 1823:2261-2273.
- Lechaue C, Augustin S, Roussel D, Sahel JA, Corral-Debrinski M (2013) Neuroglobin involvement in visual pathways through the optic nerve. *Biochim Biophys Acta* 1834:1772-1778.
- Lee JA, Beigneux A, Ahmad ST, Young SG, Gao FB (2007) ESCRT-III dysfunction causes autophagosome accumulation and neurodegeneration. *Curr Biol* 17:1561-1567.
- Lee JH, Yu WH, Kumar A, Lee S, Mohan PS, Peterhoff CM, Wolfe DM, Martinez-Vicente M, Massey AC, Sovak G, Uchiyama Y, Westaway D, Cuervo AM, Nixon RA (2010a) Lysosomal proteolysis and autophagy require presenilin 1 and are disrupted by Alzheimer-related PS1 mutations. *Cell* 141:1146-1158.
- Lee JW, Park S, Takahashi Y, Wang HG (2010b) The association of AMPK with ULK1 regulates autophagy. *PLoS One* 5:e15394.
- Lee JY, Nagano Y, Taylor JP, Lim KL, Yao TP (2010c) Disease-causing mutations in parkin impair mitochondrial ubiquitination, aggregation, and HDAC6-dependent mitophagy. *J Cell Biol* 189:671-679.
- Lee S, Sterky FH, Mourier A, Terzioglu M, Cullheim S, Olson L, Larsson NG (2012) Mitofusin 2 is necessary for striatal axonal projections of midbrain dopamine neurons. *Hum Mol Genet* 21:4827-4835.
- Lee YJ, Jeong SY, Karbowski M, Smith CL, Youle RJ (2004a) Roles of the mammalian mitochondrial fission and fusion mediators Fis1, Drp1, and Opa1 in apoptosis. *Mol Biol Cell* 15:5001-5011.
- Lee YJ, Jeong SY, Karbowski M, Smith CL, Youle RJ (2004b) Roles of the mammalian mitochondrial fission and fusion mediators Fis1, Drp1, and Opa1 in apoptosis. *Mol Biol Cell* 15:5001-5011.
- Legros F, Lombes A, Frachon P, Rojo M (2002a) Mitochondrial fusion in human cells is efficient, requires the inner membrane potential, and is mediated by mitofusins. *Mol Biol Cell* 13:4343-4354.
- Legros F, Lombes A, Frachon P, Rojo M (2002b) Mitochondrial fusion in human cells is efficient, requires the inner membrane potential, and is mediated by mitofusins. *Mol Biol Cell* 13:4343-4354.
- Lenaers G, Hamel C, Delettre C, Amati-Bonneau P, Procaccio V, Bonneau D, Reynier P, Milea D (2012) Dominant optic atrophy. *Orphanet J Rare Dis* 7:46.
- Levine B, Klionsky DJ (2004) Development by self-digestion: molecular mechanisms and biological functions of autophagy. *Dev Cell* 6:463-477.
- Li Q, Vande VC, Israelson A, Xie J, Bailey AO, Dong MQ, Chun SJ, Roy T, Winer L, Yates JR, Capaldi RA, Cleveland DW, Miller TM (2010) ALS-linked mutant superoxide dismutase 1 (SOD1) alters mitochondrial protein composition and decreases protein import. *Proc Natl Acad Sci U S A* 107:21146-21151.
- Liu X, Weaver D, Shiriha O, Hajnoczky G (2009) Mitochondrial 'kiss-and-run': interplay between mitochondrial motility and fusion-fission dynamics. *EMBO J* 28:3074-3089.
- Loson OC, Song Z, Chen H, Chan DC (2013) Fis1, Mff, MiD49, and MiD51 mediate Drp1 recruitment in mitochondrial fission. *Mol Biol Cell* 24:659-667.
- Ma H, Cai Q, Lu W, Sheng ZH, Mochida S (2009) KIF5B motor adaptor syntabulin maintains synaptic transmission in sympathetic neurons. *J Neurosci* 29:13019-13029.
- MacAskill AF, Brickley K, Stephenson FA, Kittler JT (2009) GTPase dependent recruitment of Grif-1 by Miro1 regulates mitochondrial trafficking in hippocampal neurons. *Mol Cell Neurosci* 40:301-312.
- Magrane J, Hervias I, Henning MS, Damiano M, Kawamata H, Manfredi G (2009) Mutant SOD1 in neuronal mitochondria causes toxicity and mitochondrial dynamics abnormalities. *Hum Mol Genet* 18:4552-4564.
- Mairet-Coello G, Courchet J, Pieraut S, Courchet V, Maximov A, Polleux F (2013) The CAMKK2-AMPK kinase pathway mediates the synaptotoxic effects of Abeta oligomers through Tau phosphorylation. *Neuron* 78:94-108.
- Manczak M, Reddy PH (2012) Abnormal interaction between the mitochondrial fission protein Drp1 and hyperphosphorylated tau in Alzheimer's disease neurons: implications for mitochondrial dysfunction and neuronal damage. *Hum Mol Genet* 21:2538-2547.

Mannella CA, Marko M, Penczek P, Barnard D, Frank J (1994) The internal compartmentation of rat-liver mitochondria: tomographic study using the high-voltage transmission electron microscope. *Microsc Res Tech* 27:278-283.

Marchbank NJ, Craig JE, Leek JP, Toohey M, Churchill AJ, Markham AF, Mackey DA, Toomes C, Inglehearn CF (2002) Deletion of the OPA1 gene in a dominant optic atrophy family: evidence that haploinsufficiency is the cause of disease. *J Med Genet* 39:e47.

Massieu L, Del RP, Montiel T (2001) Neurotoxicity of glutamate uptake inhibition in vivo: correlation with succinate dehydrogenase activity and prevention by energy substrates. *Neuroscience* 106:669-677.

Matsuda N, Sato S, Shiba K, Okatsu K, Saisho K, Gautier CA, Sou YS, Saiki S, Kawajiri S, Sato F, Kimura M, Komatsu M, Hattori N, Tanaka K (2010) PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. *J Cell Biol* 189:211-221.

Matsui H, Gavinio R, Asano T, Uemura N, Ito H, Taniguchi Y, Kobayashi Y, Maki T, Shen J, Takeda S, Uemura K, Yamakado H, Takahashi R (2013) PINK1 and Parkin complementarily protect dopaminergic neurons in vertebrates. *Hum Mol Genet* 22:2423-2434.

Mattenberger Y, James DI, Martinou JC (2003) Fusion of mitochondria in mammalian cells is dependent on the mitochondrial inner membrane potential and independent of microtubules or actin. *FEBS Lett* 538:53-59.

Meeusen S, DeVay R, Block J, Cassidy-Stone A, Wayson S, McCaffery JM, Nunnari J (2006) Mitochondrial inner-membrane fusion and crista maintenance requires the dynamin-related GTPase Mgm1. *Cell* 127:383-395.

Meeusen S, McCaffery JM, Nunnari J (2004) Mitochondrial fusion intermediates revealed in vitro. *Science* 305:1747-1752.

Meissner C, Lorenz H, Weihofen A, Selkoe DJ, Lemberg MK (2011) The mitochondrial intramembrane protease PARL cleaves human Pink1 to regulate Pink1 trafficking. *J Neurochem* 117:856-867.

Merkwirth C, Dargazanli S, Tatsuta T, Geimer S, Lower B, Wunderlich FT, von Kleist-Retzow JC, Waisman A, Westermann B, Langer T (2008) Prohibitins control cell proliferation and apoptosis by regulating OPA1-dependent cristae morphogenesis in mitochondria. *Genes Dev* 22:476-488.

Michiorri S, Gelmetti V, Giarda E, Lombardi F, Romano F, Marongiu R, Nerini-Molteni S, Sale P, Vago R, Arena G, Torosantucci L, Cassina L, Russo MA, Dallapiccola B, Valente EM, Casari G (2010) The Parkinson-associated protein PINK1 interacts with Beclin1 and promotes autophagy. *Cell Death Differ* 17:962-974.

Mihaylova MM, Shaw RJ (2011) The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. *Nat Cell Biol* 13:1016-1023.

Millecamps S, Gentil BJ, Gros-Louis F, Rouleau G, Julien JP (2005) Alsin is partially associated with centrosome in human cells. *Biochim Biophys Acta* 1745:84-100.

Miller KE, Sheetz MP (2004) Axonal mitochondrial transport and potential are correlated. *J Cell Sci* 117:2791-2804.

Minin AA, Kulik AV, Gyoeva FK, Li Y, Goshima G, Gelfand VI (2006) Regulation of mitochondria distribution by RhoA and formins. *J Cell Sci* 119:659-670.

Misaka T, Miyashita T, Kubo Y (2002) Primary structure of a dynamin-related mouse mitochondrial GTPase and its distribution in brain, subcellular localization, and effect on mitochondrial morphology. *J Biol Chem* 277:15834-15842.

Misko A, Jiang S, Wegorzewska I, Milbrandt J, Baloh RH (2010) Mitofusin 2 is necessary for transport of axonal mitochondria and interacts with the Miro/Milton complex. *J Neurosci* 30:4232-4240.

Misko AL, Sasaki Y, Tuck E, Milbrandt J, Baloh RH (2012) Mitofusin2 mutations disrupt axonal mitochondrial positioning and promote axon degeneration. *J Neurosci* 32:4145-4155.

Mitchell P, Moyle J (1965) Stoichiometry of proton translocation through the respiratory chain and adenosine triphosphatase systems of rat liver mitochondria. *Nature* 208:147-151.

Mizushima N (2007) Autophagy: process and function. *Genes Dev* 21:2861-2873.

Mizushima N (2010) The role of the Atg1/ULK1 complex in autophagy regulation. *Curr Opin Cell Biol* 22:132-139.

Nah J, Pyo JO, Jung S, Yoo SM, Kam TI, Chang J, Han J, An SS, Onodera T, Jung YK (2013) BECN1/Beclin1 is recruited into lipid rafts by prion to activate autophagy in response to amyloid beta 42. *Autophagy* 9.

Nakamura N, Kimura Y, Tokuda M, Honda S, Hirose S (2006) MARCH-V is a novel mitofusin 2- and Drp1-binding protein able to change mitochondrial morphology. *EMBO Rep* 7:1019-1022.

Nakazawa A, Usuda N, Matsui T, Hanai T, Matsushita S, Arai H, Sasaki H, Higuchi S (2001) Localization of calcineurin in the mature and developing retina. *J Histochem Cytochem* 49:187-195.

Narendra D, Kane LA, Hauser DN, Fearnley IM, Youle RJ (2010) p62/SQSTM1 is required for Parkin-induced mitochondrial clustering but not mitophagy; VDAC1 is dispensable for both. *Autophagy* 6:1090-1106.

Narendra D, Tanaka A, Suen DF, Youle RJ (2008) Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. *J Cell Biol* 183:795-803.

Nelson R, Famiglietti EV, Jr., Kolb H (1978) Intracellular staining reveals different levels of stratification for on- and off-center ganglion cells in cat retina. *J Neurophysiol* 41:472-483.

Niemann A, Ruegg M, La P, V, Schenone A, Suter U (2005) Ganglioside-induced differentiation associated protein 1 is a regulator of the mitochondrial network: new implications for Charcot-Marie-Tooth disease. *J Cell Biol* 170:1067-1078.

Niemann A, Wagner KM, Ruegg M, Suter U (2009) GDAP1 mutations differ in their effects on mitochondrial dynamics and apoptosis depending on the mode of inheritance. *Neurobiol Dis* 36:509-520.

Niescier RF, Chang KT, Min KT (2013) Miro, MCU, and calcium: bridging our understanding of mitochondrial movement in axons. *Front Cell Neurosci* 7:148.

Nilsson P, Loganathan K, Sekiguchi M, Matsuba Y, Hui K, Tsubuki S, Tanaka M, Iwata N, Saito T, Saido TC (2013) Abeta secretion and plaque formation depend on autophagy. *Cell Rep* 5:61-69.

Nishiyama J, Miura E, Mizushima N, Watanabe M, Yuzaki M (2007) Aberrant membranes and double-membrane structures accumulate in the axons of Atg5-null Purkinje cells before neuronal death. *Autophagy* 3:591-596.

Niu J, Yu M, Wang C, Xu Z (2012) Leucine-rich repeat kinase 2 disturbs mitochondrial dynamics via Dynamin-like protein. *J Neurochem* 122:650-658.

Nixon RA (2007) Autophagy, amyloidogenesis and Alzheimer disease. *J Cell Sci* 120:4081-4091.

Nixon RA, Wegiel J, Kumar A, Yu WH, Peterhoff C, Cataldo A, Cuervo AM (2005) Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study. *J Neuropathol Exp Neurol* 64:113-122.

Nixon RA, Yang DS (2011) Autophagy failure in Alzheimer's disease--locating the primary defect. *Neurobiol Dis* 43:38-45.

Oakley MG, Hollenbeck JJ (2001) The design of antiparallel coiled coils. *Curr Opin Struct Biol* 11:450-457.

Obashi K, Okabe S (2013) Regulation of mitochondrial dynamics and distribution by synapse position and neuronal activity in the axon. *Eur J Neurosci* 38:2350-2363.

Ohno N, Kidd GJ, Mahad D, Kiryu-Seo S, Avishai A, Komuro H, Trapp BD (2011) Myelination and axonal electrical activity modulate the distribution and motility of mitochondria at CNS nodes of Ranvier. *J Neurosci* 31:7249-7258.

Okatsu K, Saisho K, Shimanuki M, Nakada K, Shitara H, Sou YS, Kimura M, Sato S, Hattori N, Komatsu M, Tanaka K, Matsuda N (2010) p62/SQSTM1 cooperates with Parkin for perinuclear clustering of depolarized mitochondria. *Genes Cells* 15:887-900.

Olichon A, Baricault L, Gas N, Guillou E, Valette A, Belenguer P, Lenaers G (2003a) Loss of OPA1 perturbs the mitochondrial inner membrane structure and integrity, leading to cytochrome c release and apoptosis. *J Biol Chem* 278:7743-7746.

Olichon A, Baricault L, Gas N, Guillou E, Valette A, Belenguer P, Lenaers G (2003b) Loss of OPA1 perturbs the mitochondrial inner membrane structure and integrity, leading to cytochrome c release and apoptosis. *J Biol Chem* 278:7743-7746.

Olichon A, Emorine LJ, Descoins E, Pelloquin L, Brichese L, Gas N, Guillou E, Delettre C, Valette A, Hamel CP, Ducommun B, Lenaers G, Belenguer P (2002) The human dynamin-related protein OPA1 is anchored to the mitochondrial inner membrane facing the inter-membrane space. *FEBS Lett* 523:171-176.

Orth M, Tabrizi SJ, Tomlinson C, Messmer K, Korlipara LV, Schapira AH, Cooper JM (2004) G209A mutant alpha synuclein expression specifically enhances dopamine induced oxidative damage. *Neurochem Int* 45:669-676.

Osellame LD, Duchen MR (2013) Quality Control Gone Wrong: Mitochondria, Lysosomal Storage Disorders and Neurodegeneration. *Br J Pharmacol*.

Otera H, Wang C, Cleland MM, Setoguchi K, Yokota S, Youle RJ, Mihara K (2010) Mff is an essential factor for mitochondrial recruitment of Drp1 during mitochondrial fission in mammalian cells. *J Cell Biol* 191:1141-1158.

Palade GE (1952) The fine structure of mitochondria. *Anat Rec* 114:427-451.

Panov AV, Gutekunst CA, Leavitt BR, Hayden MR, Burke JR, Strittmatter WJ, Greenamyre JT (2002) Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. *Nat Neurosci* 5:731-736.

Park J, Lee SB, Lee S, Kim Y, Song S, Kim S, Bae E, Kim J, Shong M, Kim JM, Chung J (2006) Mitochondrial dysfunction in *Drosophila* PINK1 mutants is complemented by parkin. *Nature* 441:1157-1161.

Pathak D, Sepp KJ, Hollenbeck PJ (2010) Evidence that myosin activity opposes microtubule-based axonal transport of mitochondria. *J Neurosci* 30:8984-8992.

Pelloquin L, Belenguer P, Menon Y, Ducommun B (1998) Identification of a fission yeast dynamin-related protein involved in mitochondrial DNA maintenance. *Biochem Biophys Res Commun* 251:720-726.

Pereira C, Santos MS, Oliveira C (1998) Mitochondrial function impairment induced by amyloid beta-peptide on PC12 cells. *Neuroreport* 9:1749-1755.

Perkins G, Renken C, Martone ME, Young SJ, Ellisman M, Frey T (1997) Electron tomography of neuronal mitochondria: three-dimensional structure and organization of cristae and membrane contacts. *J Struct Biol* 119:260-272.

Perry VH, Henderson Z, Linden R (1983) Postnatal changes in retinal ganglion cell and optic axon populations in the pigmented rat. *J Comp Neurol* 219:356-368.

Pesch UE, Leo-Kottler B, Mayer S, Jurklies B, Kellner U, Apfelstedt-Sylla E, Zrenner E, Alexander C, Wissinger B (2001) OPA1 mutations in patients with autosomal dominant optic atrophy and evidence for semi-dominant inheritance. *Hum Mol Genet* 10:1359-1368.

Pich S, Bach D, Briones P, Liesa M, Camps M, Testar X, Palacin M, Zorzano A (2005) The Charcot-Marie-Tooth type 2A gene product, Mfn2, up-regulates fuel oxidation through expression of OXPHOS system. *Hum Mol Genet* 14:1405-1415.

Pickford F, Masliah E, Britschgi M, Lucin K, Narasimhan R, Jaeger PA, Small S, Spencer B, Rockenstein E, Levine B, Wyss-Coray T (2008) The autophagy-related protein beclin 1 shows reduced expression in early Alzheimer disease and regulates amyloid beta accumulation in mice. *J Clin Invest* 118:2190-2199.

Pilling AD, Horiuchi D, Lively CM, Saxton WM (2006) Kinesin-1 and Dynein are the primary motors for fast transport of mitochondria in *Drosophila* motor axons. *Mol Biol Cell* 17:2057-2068.

Poole AC, Thomas RE, Andrews LA, McBride HM, Whitworth AJ, Pallanck LJ (2008) The PINK1/Parkin pathway regulates mitochondrial morphology. *Proc Natl Acad Sci U S A* 105:1638-1643.

Poole AC, Thomas RE, Yu S, Vincow ES, Pallanck L (2010) The mitochondrial fusion-promoting factor mitofusin is a substrate of the PINK1/parkin pathway. *PLoS One* 5:e10054.

Praefcke GJ, McMahon HT (2004) The dynamin superfamily: universal membrane tubulation and fission molecules? *Nat Rev Mol Cell Biol* 5:133-147.

Priault M, Salin B, Schaeffer J, Vallette FM, di Rago JP, Martinou JC (2005) Impairing the bioenergetic status and the biogenesis of mitochondria triggers mitophagy in yeast. *Cell Death Differ* 12:1613-1621.

Quintero OA, DiVito MM, Adikes RC, Kortan MB, Case LB, Lier AJ, Panaretos NS, Slater SQ, Rengarajan M, Feliu M, Cheney RE (2009) Human Myo19 is a novel myosin that associates with mitochondria. *Curr Biol* 19:2008-2013.

Rakovic A, Shurkewitsch K, Seibler P, Grunewald A, Zanon A, Hagenah J, Krainc D, Klein C (2013) Phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1)-dependent ubiquitination of endogenous Parkin attenuates mitophagy: study in human primary fibroblasts and induced pluripotent stem cell-derived neurons. *J Biol Chem* 288:2223-2237.

Ravikumar B, Moreau K, Jahreiss L, Puri C, Rubinsztein DC (2010) Plasma membrane contributes to the formation of pre-autophagosomal structures. *Nat Cell Biol* 12:747-757.

Rawanduzy A, Hansen A, Hansen TW, Nedergaard M (1997) Effective reduction of infarct volume by gap junction blockade in a rodent model of stroke. *Neurosurg Focus* 2:E1.

Rintoul GL, Bennett VJ, Papaconstantinou NA, Reynolds IJ (2006) Nitric oxide inhibits mitochondrial movement in forebrain neurons associated with disruption of mitochondrial membrane potential. *J Neurochem* 97:800-806.

Rodieck RW, Watanabe M (1993) Survey of the morphology of macaque retinal ganglion cells that project to the pretectum, superior colliculus, and parvocellular laminae of the lateral geniculate nucleus. *J Comp Neurol* 338:289-303.

Rajo M, Legros F, Chateau D, Lombes A (2002) Membrane topology and mitochondrial targeting of mitofusins, ubiquitous mammalian homologs of the transmembrane GTPase Fzo. *J Cell Sci* 115:1663-1674.

- Rosenstock TR, de Brito OM, Lombardi V, Louros S, Ribeiro M, Almeida S, Ferreira IL, Oliveira CR, Rego AC (2011) FK506 ameliorates cell death features in Huntington's disease striatal cell models. *Neurochem Int* 59:600-609.
- Rui Y, Tiwari P, Xie Z, Zheng JQ (2006) Acute impairment of mitochondrial trafficking by beta-amyloid peptides in hippocampal neurons. *J Neurosci* 26:10480-10487.
- Russo GJ, Louie K, Wellington A, Macleod GT, Hu F, Panchumarthi S, Zinsmaier KE (2009) Drosophila Miro is required for both anterograde and retrograde axonal mitochondrial transport. *J Neurosci* 29:5443-5455.
- Saito H (1983) Pharmacological and morphological differences between X- and Y-type ganglion cells in the cat's retina. *Vision Res* 23:1299-1308.
- Sanders MJ, Grondin PO, Hegarty BD, Snowden MA, Carling D (2007) Investigating the mechanism for AMP activation of the AMP-activated protein kinase cascade. *Biochem J* 403:139-148.
- Santel A (2006) Get the balance right: mitofusins roles in health and disease. *Biochim Biophys Acta* 1763:490-499.
- Santel A, Frank S, Gaume B, Herrler M, Youle RJ, Fuller MT (2003a) Mitofusin-1 protein is a generally expressed mediator of mitochondrial fusion in mammalian cells. *J Cell Sci* 116:2763-2774.
- Santel A, Frank S, Gaume B, Herrler M, Youle RJ, Fuller MT (2003b) Mitofusin-1 protein is a generally expressed mediator of mitochondrial fusion in mammalian cells. *J Cell Sci* 116:2763-2774.
- Santel A, Fuller MT (2001a) Control of mitochondrial morphology by a human mitofusin. *J Cell Sci* 114:867-874.
- Santel A, Fuller MT (2001b) Control of mitochondrial morphology by a human mitofusin. *J Cell Sci* 114:867-874.
- Sarraf SA, Raman M, Guarani-Pereira V, Sowa ME, Huttlin EL, Gygi SP, Harper JW (2013) Landscape of the PARKIN-dependent ubiquitylome in response to mitochondrial depolarization. *Nature* 496:372-376.
- Sarzi E, Angebault C, Seveno M, Gueguen N, Chaix B, Bielicki G, Boddaert N, Matusset-Bonnefont AL, Cazevieville C, Rigau V, Renou JP, Wang J, Delettre C, Brabet P, Puel JL, Hamel CP, Reynier P, Lenaers G (2012) The human OPA1delTTAG mutation induces premature age-related systemic neurodegeneration in mouse. *Brain* 135:3599-3613.
- Sasaki S, Iwata M (2007) Mitochondrial alterations in the spinal cord of patients with sporadic amyotrophic lateral sclerosis. *J Neuropathol Exp Neurol* 66:10-16.
- Sasaki S, Warita H, Murakami T, Shibata N, Komori T, Abe K, Kobayashi M, Iwata M (2005) Ultrastructural study of aggregates in the spinal cord of transgenic mice with a G93A mutant SOD1 gene. *Acta Neuropathol* 109:247-255.
- Satoh M, Hamamoto T, Seo N, Kagawa Y, Endo H (2003) Differential sublocalization of the dynamin-related protein OPA1 isoforms in mitochondria. *Biochem Biophys Res Commun* 300:482-493.
- Sau D, Rusmini P, Crippa V, Onesto E, Bolzoni E, Ratti A, Poletti A (2011) Dysregulation of axonal transport and motorneuron diseases. *Biol Cell* 103:87-107.
- Schapira AH, Cooper JM, Dexter D, Clark JB, Jenner P, Marsden CD (1990) Mitochondrial complex I deficiency in Parkinson's disease. *J Neurochem* 54:823-827.
- Schatz G (2007) The magic garden. *Annu Rev Biochem* 76:673-678.
- Schwarzer C, Barnikol-Watanabe S, Thinnies FP, Hilschmann N (2002) Voltage-dependent anion-selective channel (VDAC) interacts with the dynein light chain Tctex1 and the heat-shock protein PBP74. *Int J Biochem Cell Biol* 34:1059-1070.
- Scorrano L, Ashiya M, Buttle K, Weiler S, Oakes SA, Mannella CA, Korsmeyer SJ (2002) A Distinct Pathway Remodels Mitochondrial Cristae and Mobilizes Cytochrome c during Apoptosis. *Dev Cell* 2:55-67.
- Scorrano L, Oakes SA, Opferman JT, Cheng EH, Sorcinelli MD, Pozzan T, Korsmeyer SJ (2003) BAX and BAK regulation of endoplasmic reticulum Ca²⁺: a control point for apoptosis. *Science* 300:135-139.
- Scorrano L, Petronilli V, Bernardi P (1997) On the voltage dependence of the mitochondrial permeability transition pore. A critical appraisal. *J Biol Chem* 272:12295-12299.
- Seibler P, Graziotto J, Jeong H, Simunovic F, Klein C, Krainc D (2011) Mitochondrial Parkin recruitment is impaired in neurons derived from mutant PINK1 induced pluripotent stem cells. *J Neurosci* 31:5970-5976.
- Sernagor E, Eglens SJ, Wong RO (2001) Development of retinal ganglion cell structure and function. *Prog Retin Eye Res* 20:139-174.

Sesaki H, Southard SM, Yaffe MP, Jensen RE (2003) Mgm1p, a dynamin-related GTPase, is essential for fusion of the mitochondrial outer membrane. *Mol Biol Cell* 14:2342-2356.

Shan X, Chiang PM, Price DL, Wong PC (2010) Altered distributions of Gemini of coiled bodies and mitochondria in motor neurons of TDP-43 transgenic mice. *Proc Natl Acad Sci U S A* 107:16325-16330.

Shavali S, Sens DA (2008) Synergistic neurotoxic effects of arsenic and dopamine in human dopaminergic neuroblastoma SH-SY5Y cells. *Toxicol Sci* 102:254-261.

Sheng ZH, Cai Q (2012) Mitochondrial transport in neurons: impact on synaptic homeostasis and neurodegeneration. *Nat Rev Neurosci* 13:77-93.

Shi SH, Jan LY, Jan YN (2003) Hippocampal neuronal polarity specified by spatially localized mPar3/mPar6 and PI 3-kinase activity. *Cell* 112:63-75.

Shin JH, Ko HS, Kang H, Lee Y, Lee YI, Pletinkova O, Troconso JC, Dawson VL, Dawson TM (2011) PARIS (ZNF746) repression of PGC-1alpha contributes to neurodegeneration in Parkinson's disease. *Cell* 144:689-702.

Shirendeb UP, Calkins MJ, Manczak M, Anekonda V, Dufour B, McBride JL, Mao P, Reddy PH (2012) Mutant huntingtin's interaction with mitochondrial protein Drp1 impairs mitochondrial biogenesis and causes defective axonal transport and synaptic degeneration in Huntington's disease. *Hum Mol Genet* 21:406-420.

Siintola E, Partanen S, Stromme P, Haapanen A, Haltia M, Maehlen J, Lehesjoki AE, Tyynela J (2006) Cathepsin D deficiency underlies congenital human neuronal ceroid-lipofuscinosis. *Brain* 129:1438-1445.

Singh SB, Ornatowski W, Vergne I, Naylor J, Delgado M, Roberts E, Ponpuak M, Master S, Pilli M, White E, Komatsu M, Deretic V (2010) Human IRGM regulates autophagy and cell-autonomous immunity functions through mitochondria. *Nat Cell Biol* 12:1154-1165.

SJOSTRAND FS (1953) Electron microscopy of mitochondria and cytoplasmic double membranes. *Nature* 171:30-32.

Smirnova E, Griparic L, Shurland DL, van der Bliek AM (2001a) Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells. *Mol Biol Cell* 12:2245-2256.

Smirnova E, Griparic L, Shurland DL, van der Bliek AM (2001b) Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells. *Mol Biol Cell* 12:2245-2256.

Song W, Chen J, Petrilli A, Liot G, Klinglmayr E, Zhou Y, Poquiz P, Tjong J, Pouladi MA, Hayden MR, Masliah E, Ellisman M, Rouiller I, Schwarzenbacher R, Bossy B, Perkins G, Bossy-Wetzel E (2011) Mutant huntingtin binds the mitochondrial fission GTPase dynamin-related protein-1 and increases its enzymatic activity. *Nat Med* 17:377-382.

Song Z, Chen H, Fiket M, Alexander C, Chan DC (2007) OPA1 processing controls mitochondrial fusion and is regulated by mRNA splicing, membrane potential, and Yme1L. *J Cell Biol* 178:749-755.

Soyombo AA, Tjon-Kon-Sang S, Rbaibi Y, Bashllari E, Bisceglia J, Muallem S, Kiselyov K (2006) TRP-ML1 regulates lysosomal pH and acidic lysosomal lipid hydrolytic activity. *J Biol Chem* 281:7294-7301.

Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M (1998) alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. *Proc Natl Acad Sci U S A* 95:6469-6473.

St-Pierre J, Brand MD, Boutilier RG (2000) Mitochondria as ATP consumers: cellular treason in anoxia. *Proc Natl Acad Sci U S A* 97:8670-8674.

Stanford LR, Sherman SM (1984) Structure/function relationships of retinal ganglion cells in the cat. *Brain Res* 297:381-386.

Stein SC, Woods A, Jones NA, Davison MD, Carling D (2000) The regulation of AMP-activated protein kinase by phosphorylation. *Biochem J* 345 Pt 3:437-443.

Stemmer PM, Klee CB (1994) Dual calcium ion regulation of calcineurin by calmodulin and calcineurin B. *Biochemistry* 33:6859-6866.

Stephenson FA (2013) Revisiting the TRAK Family of Proteins as Mediators of GABA Receptor Trafficking. *Neurochem Res*.

Sterky FH, Lee S, Wibom R, Olson L, Larsson NG (2011) Impaired mitochondrial transport and Parkin-independent degeneration of respiratory chain-deficient dopamine neurons in vivo. *Proc Natl Acad Sci U S A* 108:12937-12942.

Stokin GB, Lillo C, Falzone TL, Brusch RG, Rockenstein E, Mount SL, Raman R, Davies P, Masliah E, Williams DS, Goldstein LS (2005) Axonopathy and transport deficits early in the pathogenesis of Alzheimer's disease. *Science* 307:1282-1288.

Stowers RS, Megeath LJ, Gorska-Andrzejak J, Meinertzhagen IA, Schwarz TL (2002) Axonal transport of mitochondria to synapses depends on Milton, a novel Drosophila protein. *Neuron* 36:1063-1077.

- Strauss M, Hofhaus G, Schroder RR, Kuhlbrandt W (2008) Dimer ribbons of ATP synthase shape the inner mitochondrial membrane. *EMBO J* 27:1154-1160.
- Sturmer K, Baumann O, Walz B (1995) Actin-dependent light-induced translocation of mitochondria and ER cisternae in the photoreceptor cells of the locust *Schistocerca gregaria*. *J Cell Sci* 108 (Pt 6):2273-2283.
- Sung JY, Engmann O, Teylan MA, Nairn AC, Greengard P, Kim Y (2008) WAVE1 controls neuronal activity-induced mitochondrial distribution in dendritic spines. *Proc Natl Acad Sci U S A* 105:3112-3116.
- Taguchi N, Ishihara N, Jofuku A, Oka T, Mihara K (2007) Mitotic phosphorylation of dynamin-related GTPase Drp1 participates in mitochondrial fission. *J Biol Chem* 282:11521-11529.
- Tan JM, Wong ES, Kirkpatrick DS, Pletnikova O, Ko HS, Tay SP, Ho MW, Troncoso J, Gygi SP, Lee MK, Dawson VL, Dawson TM, Lim KL (2008) Lysine 63-linked ubiquitination promotes the formation and autophagic clearance of protein inclusions associated with neurodegenerative diseases. *Hum Mol Genet* 17:431-439.
- Tanaka A, Cleland MM, Xu S, Narendra DP, Suen DF, Karbowski M, Youle RJ (2010a) Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. *J Cell Biol* 191:1367-1380.
- Tanaka A, Cleland MM, Xu S, Narendra DP, Suen DF, Karbowski M, Youle RJ (2010b) Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. *J Cell Biol* 191:1367-1380.
- Tanaka K, Sugiura Y, Ichishita R, Mihara K, Oka T (2011) KLP6: a newly identified kinesin that regulates the morphology and transport of mitochondria in neuronal cells. *J Cell Sci* 124:2457-2465.
- Tanaka Y, Kanai Y, Okada Y, Nonaka S, Takeda S, Harada A, Hirokawa N (1998) Targeted disruption of mouse conventional kinesin heavy chain, kif5B, results in abnormal perinuclear clustering of mitochondria. *Cell* 93:1147-1158.
- Tavazoie SF, Alvarez VA, Ridenour DA, Kwiatkowski DJ, Sabatini BL (2005) Regulation of neuronal morphology and function by the tumor suppressors Tsc1 and Tsc2. *Nat Neurosci* 8:1727-1734.
- Thornton C, Bright NJ, Sastre M, Muckett PJ, Carling D (2011) AMP-activated protein kinase (AMPK) is a tau kinase, activated in response to amyloid beta-peptide exposure. *Biochem J* 434:503-512.
- Tomoda T, Bhatt RS, Kuroyanagi H, Shirasawa T, Hatten ME (1999) A mouse serine/threonine kinase homologous to *C. elegans* UNC51 functions in parallel fiber formation of cerebellar granule neurons. *Neuron* 24:833-846.
- Tondera D, Czaderna F, Paulick K, Schwarzer R, Kaufmann J, Santel A (2005) The mitochondrial protein MTP18 contributes to mitochondrial fission in mammalian cells. *J Cell Sci* 118:3049-3059.
- Tondera D, Santel A, Schwarzer R, Dames S, Giese K, Klippel A, Kaufmann J (2004) Knockdown of MTP18, a novel phosphatidylinositol 3-kinase-dependent protein, affects mitochondrial morphology and induces apoptosis. *J Biol Chem* 279:31544-31555.
- Trushina E, Nemutlu E, Zhang S, Christensen T, Camp J, Mesa J, Siddiqui A, Tamura Y, Sesaki H, Wengenack TM, Dzeja PP, Poduslo JF (2012) Defects in mitochondrial dynamics and metabolomic signatures of evolving energetic stress in mouse models of familial Alzheimer's disease. *PLoS One* 7:e32737.
- Turnley AM, Stapleton D, Mann RJ, Witters LA, Kemp BE, Bartlett PF (1999) Cellular distribution and developmental expression of AMP-activated protein kinase isoforms in mouse central nervous system. *J Neurochem* 72:1707-1716.
- Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, Stiles L, Haigh SE, Katz S, Las G, Alroy J, Wu M, Py BF, Yuan J, Deeney JT, Corkey BE, Shirihai OS (2008) Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *EMBO J* 27:433-446.
- Twig G, Liu X, Liesa M, Wikstrom JD, Molina AJ, Las G, Yaniv G, Hajnoczky G, Shirihai OS (2010) Biophysical properties of mitochondrial fusion events in pancreatic beta-cells and cardiac cells unravel potential control mechanisms of its selectivity. *Am J Physiol Cell Physiol* 299:C477-C487.
- Valente EM, et al. (2004) Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science* 304:1158-1160.
- Van Laar VS, Arnold B, Cassady SJ, Chu CT, Burton EA, Berman SB (2011) Bioenergetics of neurons inhibit the translocation response of Parkin following rapid mitochondrial depolarization. *Hum Mol Genet* 20:927-940.
- van d, V, Griffith J, Reggiori F (2010) Exit from the Golgi is required for the expansion of the autophagosomal phagophore in yeast *Saccharomyces cerevisiae*. *Mol Biol Cell* 21:2270-2284.
- Van HC, Cornelissen T, Vandenbergh W (2011) Ambra1: a Parkin-binding protein involved in mitophagy. *Autophagy* 7:1555-1556.

van SM, Mikhaylova M, Lipka J, Schlager MA, van den Heuvel DJ, Kuijpers M, Wulf PS, Keijzer N, Demmers J, Kapitein LC, Jaarsma D, Gerritsen HC, Akhmanova A, Hoogenraad CC (2013) TRAK/Milton motor-adaptor proteins steer mitochondrial trafficking to axons and dendrites. *Neuron* 77:485-502.

Vande VC, Miller TM, Cashman NR, Cleveland DW (2008) Selective association of misfolded ALS-linked mutant SOD1 with the cytoplasmic face of mitochondria. *Proc Natl Acad Sci U S A* 105:4022-4027.

Vazquez P, Arroba AI, Cecconi F, de la Rosa EJ, Boya P, de PF (2012) Atg5 and Ambra1 differentially modulate neurogenesis in neural stem cells. *Autophagy* 8:187-199.

Votruba M, Moore AT, Bhattacharya SS (1997) Genetic refinement of dominant optic atrophy (OPA1) locus to within a 2 cM interval of chromosome 3q. *J Med Genet* 34:117-121.

Wang H, Lim PJ, Karbowski M, Monteiro MJ (2009a) Effects of overexpression of huntingtin proteins on mitochondrial integrity. *Hum Mol Genet* 18:737-752.

Wang W, Li L, Lin WL, Dickson DW, Petrucelli L, Zhang T, Wang X (2013) The ALS disease-associated mutant TDP-43 impairs mitochondrial dynamics and function in motor neurons. *Hum Mol Genet* 22:4706-4719.

Wang X, Petrie TG, Liu Y, Liu J, Fujioka H, Zhu X (2012a) Parkinson's disease-associated DJ-1 mutations impair mitochondrial dynamics and cause mitochondrial dysfunction. *J Neurochem* 121:830-839.

Wang X, Schwarz TL (2009a) Imaging axonal transport of mitochondria. *Methods Enzymol* 457:319-333.

Wang X, Schwarz TL (2009b) The mechanism of Ca²⁺-dependent regulation of kinesin-mediated mitochondrial motility. *Cell* 136:163-174.

Wang X, Su B, Lee HG, Li X, Perry G, Smith MA, Zhu X (2009b) Impaired balance of mitochondrial fission and fusion in Alzheimer's disease. *J Neurosci* 29:9090-9103.

Wang X, Su B, Siedlak SL, Moreira PI, Fujioka H, Wang Y, Casadesus G, Zhu X (2008) Amyloid-beta overproduction causes abnormal mitochondrial dynamics via differential modulation of mitochondrial fission/fusion proteins. *Proc Natl Acad Sci U S A* 105:19318-19323.

Wang X, Winter D, Ashrafi G, Schlehe J, Wong YL, Selkoe D, Rice S, Steen J, LaVoie MJ, Schwarz TL (2011) PINK1 and Parkin target Miro for phosphorylation and degradation to arrest mitochondrial motility. *Cell* 147:893-906.

Wang X, Yan MH, Fujioka H, Liu J, Wilson-Delfosse A, Chen SG, Perry G, Casadesus G, Zhu X (2012b) LRRK2 regulates mitochondrial dynamics and function through direct interaction with DLP1. *Hum Mol Genet* 21:1931-1944.

Wartosch L, Stauber T (2010) A role for chloride transport in lysosomal protein degradation. *Autophagy* 6:158-159.

Wassle H, Boycott BB (1991) Functional architecture of the mammalian retina. *Physiol Rev* 71:447-480.

Wassle H, Peichl L, Boycott BB (1983) A spatial analysis of on- and off-ganglion cells in the cat retina. *Vision Res* 23:1151-1160.

Webber E, Li L, Chin LS (2008) Hypertonia-associated protein Trak1 is a novel regulator of endosome-to-lysosome trafficking. *J Mol Biol* 382:638-651.

Wei Y, Pattingre S, Sinha S, Bassik M, Levine B (2008) JNK1-mediated phosphorylation of Bcl-2 regulates starvation-induced autophagy. *Mol Cell* 30:678-688.

White KE, Davies VJ, Hogan VE, Piechota MJ, Nichols PP, Turnbull DM, Votruba M (2009) OPA1 deficiency associated with increased autophagy in retinal ganglion cells in a murine model of dominant optic atrophy. *Invest Ophthalmol Vis Sci* 50:2567-2571.

Williams PA, Piechota M, von RC, Taylor E, Morgan JE, Votruba M (2012) Opa1 is essential for retinal ganglion cell synaptic architecture and connectivity. *Brain* 135:493-505.

Winder DG, Sweatt JD (2001) Roles of serine/threonine phosphatases in hippocampal synaptic plasticity. *Nat Rev Neurosci* 2:461-474.

Winzeler A, Wang JT (2013) Purification and culture of retinal ganglion cells from rodents. *Cold Spring Harb Protoc* 2013:643-652.

Wong ED, Wagner JA, Scott SV, Okreglak V, Holewinske TJ, Cassidy-Stone A, Nunnari J (2003) The intramitochondrial dynamin-related GTPase, Mgm1p, is a component of a protein complex that mediates mitochondrial fusion. *J Cell Biol* 160:303-311.

Woods A, Dickerson K, Heath R, Hong SP, Momcilovic M, Johnstone SR, Carlson M, Carling D (2005) Ca²⁺/calmodulin-dependent protein kinase kinase-beta acts upstream of AMP-activated protein kinase in mammalian cells. *Cell Metab* 2:21-33.

Wu Y, Song P, Xu J, Zhang M, Zou MH (2007) Activation of protein phosphatase 2A by palmitate inhibits AMP-activated protein kinase. *J Biol Chem* 282:9777-9788.

- Xie M, Zhang D, Dyck JR, Li Y, Zhang H, Morishima M, Mann DL, Taffet GE, Baldini A, Khoury DS, Schneider MD (2006) A pivotal role for endogenous TGF-beta-activated kinase-1 in the LKB1/AMP-activated protein kinase energy-sensor pathway. *Proc Natl Acad Sci U S A* 103:17378-17383.
- Xu YF, Gendron TF, Zhang YJ, Lin WL, D'Alton S, Sheng H, Casey MC, Tong J, Knight J, Yu X, Rademakers R, Boylan K, Hutton M, McGowan E, Dickson DW, Lewis J, Petrucelli L (2010) Wild-type human TDP-43 expression causes TDP-43 phosphorylation, mitochondrial aggregation, motor deficits, and early mortality in transgenic mice. *J Neurosci* 30:10851-10859.
- Xu YF, Zhang YJ, Lin WL, Cao X, Stetler C, Dickson DW, Lewis J, Petrucelli L (2011) Expression of mutant TDP-43 induces neuronal dysfunction in transgenic mice. *Mol Neurodegener* 6:73.
- Yamagata M, Sanes JR (1995a) Lamina-specific cues guide outgrowth and arborization of retinal axons in the optic tectum. *Development* 121:189-200.
- Yamagata M, Sanes JR (1995b) Target-independent diversification and target-specific projection of chemically defined retinal ganglion cell subsets. *Development* 121:3763-3776.
- Yamaguchi R, Lartigue L, Perkins G, Scott RT, Dixit A, Kushnareva Y, Kuwana T, Ellisman MH, Newmeyer DD (2008) Opa1-mediated cristae opening is Bax/Bak and BH3 dependent, required for apoptosis, and independent of Bak oligomerization. *Mol Cell* 31:557-569.
- Yang DS, Stavrides P, Mohan PS, Kaushik S, Kumar A, Ohno M, Schmidt SD, Wesson D, Bandyopadhyay U, Jiang Y, Pawlik M, Peterhoff CM, Yang AJ, Wilson DA, St George-Hyslop P, Westaway D, Mathews PM, Levy E, Cuervo AM, Nixon RA (2011) Reversal of autophagy dysfunction in the TgCRND8 mouse model of Alzheimer's disease ameliorates amyloid pathologies and memory deficits. *Brain* 134:258-277.
- Yang SA, Klee C (2002) Study of calcineurin structure by limited proteolysis. *Methods Mol Biol* 172:317-334.
- Yang Y, Ouyang Y, Yang L, Beal MF, McQuibban A, Vogel H, Lu B (2008) Pink1 regulates mitochondrial dynamics through interaction with the fission/fusion machinery. *Proc Natl Acad Sci U S A* 105:7070-7075.
- Yang Z, Klionsky DJ (2009) An overview of the molecular mechanism of autophagy. *Curr Top Microbiol Immunol* 335:1-32.
- Yao J, Irwin RW, Zhao L, Nilsen J, Hamilton RT, Brinton RD (2009) Mitochondrial bioenergetic deficit precedes Alzheimer's pathology in female mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A* 106:14670-14675.
- Yla-Anttila P, Vihinen H, Jokitalo E, Eskelinen EL (2009) 3D tomography reveals connections between the phagophore and endoplasmic reticulum. *Autophagy* 5:1180-1185.
- Yonashiro R, Ishido S, Kyo S, Fukuda T, Goto E, Matsuki Y, Ohmura-Hoshino M, Sada K, Hotta H, Yamamura H, Inatome R, Yanagi S (2006) A novel mitochondrial ubiquitin ligase plays a critical role in mitochondrial dynamics. *EMBO J* 25:3618-3626.
- Yoon Y, Krueger EW, Oswald BJ, McNiven MA (2003) The mitochondrial protein hFis1 regulates mitochondrial fission in mammalian cells through an interaction with the dynamin-like protein DLP1. *Mol Cell Biol* 23:5409-5420.
- Yoon Y, Pitts KR, McNiven MA (2001) Mammalian dynamin-like protein DLP1 tubulates membranes. *Mol Biol Cell* 12:2894-2905.
- Yu DY, Cringle SJ (2001) Oxygen distribution and consumption within the retina in vascularised and avascular retinas and in animal models of retinal disease. *Prog Retin Eye Res* 20:175-208.
- Yu WH, Cuervo AM, Kumar A, Peterhoff CM, Schmidt SD, Lee JH, Mohan PS, Mercken M, Farmery MR, Tjernberg LO, Jiang Y, Duff K, Uchiyama Y, Naslund J, Mathews PM, Cataldo AM, Nixon RA (2005) Macroautophagy--a novel Beta-amyloid peptide-generating pathway activated in Alzheimer's disease. *J Cell Biol* 171:87-98.
- Zanelli SA, Trimmer PA, Solenski NJ (2006) Nitric oxide impairs mitochondrial movement in cortical neurons during hypoxia. *J Neurochem* 97:724-736.
- Zanna C, Ghelli A, Porcelli AM, Karbowski M, Youle RJ, Schimpf S, Wissinger B, Pinti M, Cossarizza A, Vidoni S, Valentino ML, Rugolo M, Carelli V (2008) OPA1 mutations associated with dominant optic atrophy impair oxidative phosphorylation and mitochondrial fusion. *Brain* 131:352-367.
- Zhang CL, Ho PL, Kintner DB, Sun D, Chiu SY (2010) Activity-dependent regulation of mitochondrial motility by calcium and Na/K-ATPase at nodes of Ranvier of myelinated nerves. *J Neurosci* 30:3555-3566.
- Zheng L, Terman A, Hallbeck M, Dehvari N, Cowburn RF, Benedikz E, Kagedal K, Cedazo-Minguez A, Marcusson J (2011) Macroautophagy-generated increase of lysosomal amyloid beta-protein mediates oxidant-induced apoptosis of cultured neuroblastoma cells. *Autophagy* 7:1528-1545.
- Zheng Z, Diamond MI (2012) Huntington disease and the huntingtin protein. *Prog Mol Biol Transl Sci* 107:189-214.

Zhu YB, Sheng ZH (2011) Increased axonal mitochondrial mobility does not slow amyotrophic lateral sclerosis (ALS)-like disease in mutant SOD1 mice. *J Biol Chem* 286:23432-23440.

Ziviani E, Tao RN, Whitworth AJ (2010a) *Drosophila* parkin requires PINK1 for mitochondrial translocation and ubiquitinates mitofusin. *Proc Natl Acad Sci U S A* 107:5018-5023.

Ziviani E, Tao RN, Whitworth AJ (2010b) *Drosophila* parkin requires PINK1 for mitochondrial translocation and ubiquitinates mitofusin. *Proc Natl Acad Sci U S A* 107:5018-5023.

Zuchner S, De JP, Jordanova A, Claeys KG, Guergueltcheva V, Cherninkova S, Hamilton SR, Van SG, Krajewski KM, Stajich J, Tournev I, Verhoeven K, Langerhorst CT, de VM, Baas F, Bird T, Timmerman V, Shy M, Vance JM (2006) Axonal neuropathy with optic atrophy is caused by mutations in mitofusin 2. *Ann Neurol* 59:276-281.

Zuchner S, et al. (2004) Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. *Nat Genet* 36:449-451.

Zunino R, Schauss A, Rippstein P, Andrade-Navarro M, McBride HM (2007) The SUMO protease SENP5 is required to maintain mitochondrial morphology and function. *J Cell Sci* 120:1178-1188.