ANTIOXIDANTS & REDOX SIGNALING
Volume 10, Number 3, 2008

DOI: 10.1089/ars.2007.1934

Forum Review

Mitofusin 2: A Mitochondria-Shaping Protein with Signaling Roles Beyond Fusion

OLGA MARTINS DE BRITO and LUCA SCORRANO

ABSTRACT

Mitochondria are central organelles in metabolism, signal transduction, and programmed cell death. To meet their diverse functional demands, their shape is strictly regulated by a growing family of proteins that impinge on fission and fusion of the organelle. Mitochondrial fusion depends on Mitofusin (Mfn) 1 and 2, two integral outer-membrane proteins. Although MFN1 seems primarily involved in the regulation of the docking and fusion of the organelle, mounting evidence is implicating MFN2 in multiple signaling pathways not restricted to the regulation of mitochondrial shape. Here we review data supporting a role for this mitochondria-shaping protein beyond fusion, in regulating mitochondrial metabolism, apoptosis, shape of other organelles, and even progression through cell cycle. In conclusion, MFN2 appears a multifunctional protein whose biologic function is not restricted to the regulation of mitochondrial shape. *Antioxid. Redox Signal.* 10, 621–633.

INTRODUCTION

Mitochondria produce ATP, the essential intermediate for the energetic demands of the cell. The products of the breakdown of carbohydrates and fatty acids enter the tricarboxylic acid (TCA) cycle in the mitochondrial matrix, where they produce reducing equivalents (NADH and FADH) that will be used by the respiratory chain. During oxidative phosphorylation, ATP synthesis results from the transfer of four electrons to O_2, giving rise to two molecules of H_2O. However, a small pool of O_2 is reduced only partially in a one-electron reaction that leads to the formation of the reactive oxygen species (ROS) superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2).

Another source of destructive free radicals in the cell is peroxynitrite ($ONOO^-$), which results from the reaction between the neurotransmitter NO and the superoxide ion $O_2^{\cdot-}$ (93). These agents together produce highly reactive singlet oxygen, hydroxyl radicals, and peroxynitrite that can attack proteins, lipids, and DNA. Free radicals are believed to be involved in the process of aging and in disease states like Alzheimer's, Parkinson's, and Huntington's disease, as well as amyotrophic lateral sclerosis, stroke, diabetes, and obesity. Mitochondrial metabolism places these organelles in the spotlight in the pathogenesis of a number of degenerative diseases in which ROS are believed to play a causative role.

The precise mechanism by which ROS production is increased under these and other pathologic conditions is not well understood. Recently, a role for mitochondrial shape changes in determining ROS production was proposed by the group of Y. Yoon (98), suggesting for the first time that factors other than mitochondrial metabolism *per se* could have a role in the pathogenesis of ROS-related diseases. These results extended the role of mitochondrial morphology beyond the control of apoptosis, Ca^{2+} signaling, cell patterning, and migration, which were recognized in the last few years as cellular functions influenced by the shape of this organelle. In this review, we analyze the role of mitochondria-shaping proteins as potential regulators of mitochondrial metabolism and therefore of ROS production.

Mammalian mitochondria-shaping proteins

Mitochondrial morphology in living cells is very heterogeneous and can range from small spheres to interconnected tubules (8). The morphologic heterogeneity of this organelle

Dulbecco-Telethon Institute, Venetian Institute of Molecular Medicine, Padua, Italy.

results from its ability to undergo fusion and fission. Individual mitochondrial tubules continuously move back and forth along their long axes on radial tracks. Two mitochondrial tubules can seldom encounter each other and fuse, end to end or head to side. Conversely, tubules can also undergo fission events, giving rise to two or more mitochondrial units (8). Moreover, mitochondrial ultrastructure is also extremely complex, with several subcompartments—cristae, inner boundary membrane, intermembrane space, outer membrane—whose shape is also tightly regulated (31). A cartoon of the mitochondrial shapes in the cell, as well as of the ultrastructure of the organelle, is presented in Fig. 1. As outlined earlier, growing evidence indicates that mitochondrial morphology is critical for cell function, and changes in mitochondrial shape have been related to apoptotic cell death, development, neurodegeneration, calcium signaling, cell division, and ROS production (12).

The shape of mitochondria depends on a growing set of proteins that regulate the fusion–fission equilibrium of the organelle. Members of this family include dynamin-related proteins, large mechanoenzymatic GTPases involved in the tubulation and severing of biologic membranes (73), as well as other "nonconventional" proteins whose molecular function is less characterized, that regulate these processes or directly participate in them *via* a yet-unclear mechanism. A cartoon of the "core" mitochondria-shaping proteins, highlighting their functional domains, is presented in Fig. 2.

Pro-fusion mitochondria-shaping proteins

Proteins involved in the regulation of mitochondrial fusion and fission in mammalian cells have been identified only recently. The first identified mediator of mitochondrial fusion was the *Drosophila melanogaster* Fuzzy onion 1 protein (Fzo1p), a large transmembrane GTPase of the outer mitochondrial membrane, required for the formation of the giant mitochondrial derivative during spermatogenesis. Fzo1p has two homologues in mammals, MFN1 and MFN2 (78), which both control mitochondrial fusion. They possess an N-terminal GTPase domain, two transmembrane domains spanning the outer mitochondrial membrane and two regions crucial for protein–protein interaction (55, 78). These two proteins are highly similar, as indicated by the Clustal alignment presented in Fig. 3, yet as will become clear later, they probably fulfil different roles in cell physiology. Deletion of either Mfn impairs embryonic development in the mouse at different stages (13). In particular, MFN2 is required for placentation, whereas MFN1 plays a specific role during embryogenesis, as shown by conditional deletion of the alleles (14).

The only dynamin-like GTPase so far identified in the IM is OPA1, mutated in dominant optic atrophy (DOA), the most

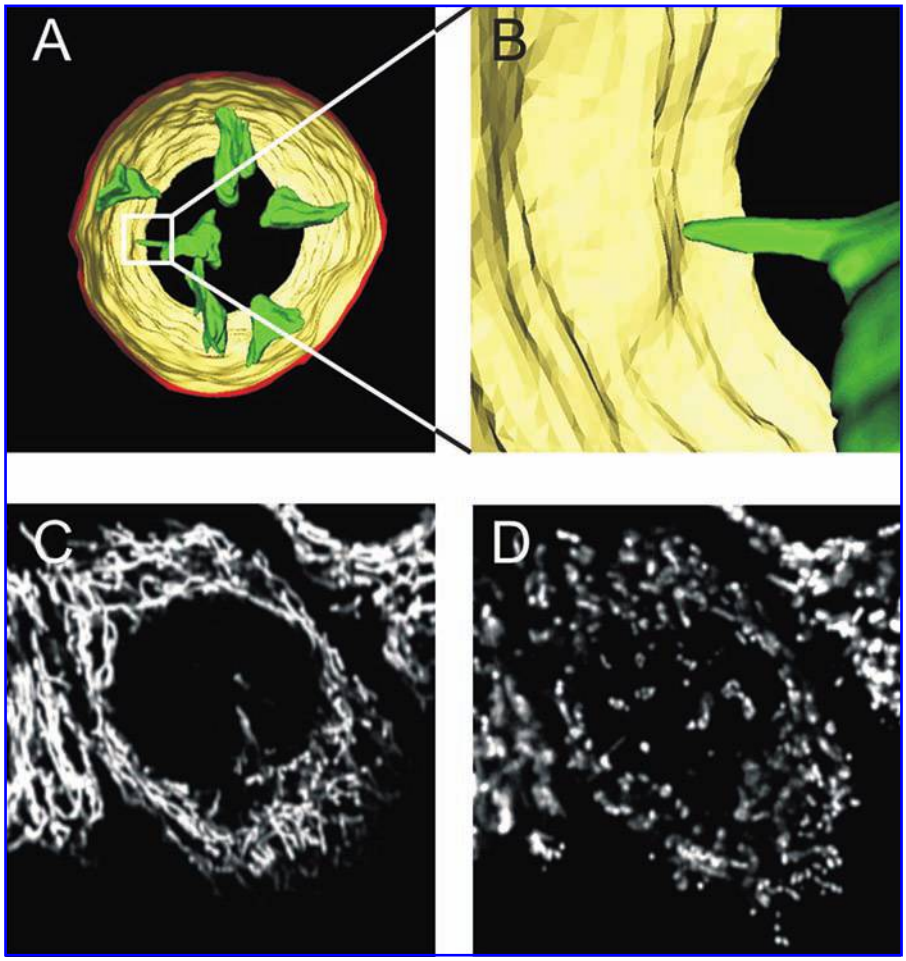

FIG. 1. Mitochondrial ultrastructure and shape. The images depict the individual mitochondrial ultrastructure determined by electron tomography (**A, B**) as well as its fused (**C**) or fragmented (**D**) morphology in the cytosol of an HeLa cell expressing mitochondrially targeted dsRED. In (**A, B**), the outer membrane is in red, the inner boundary membrane in pale yellow, and the cristae in green. For further details on the tomogram, see ref. 82. The boxed area in (**A**) is magnified in (**B**) to show the so-called tubular cristae junction.

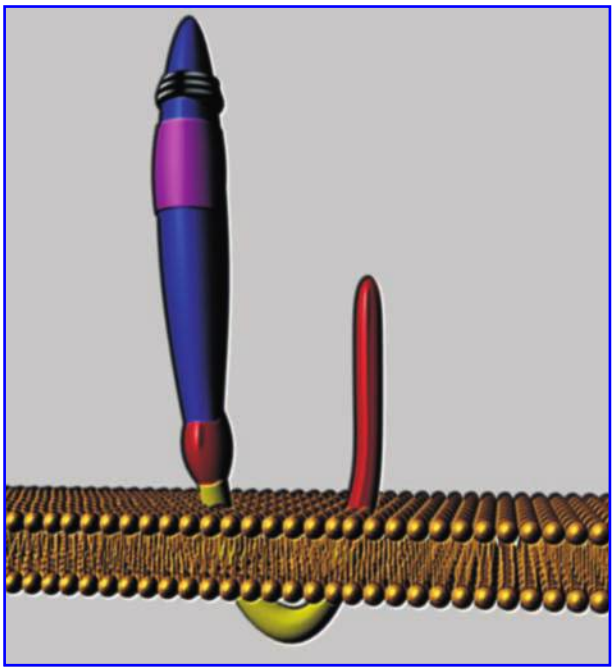

FIG. 2. "Core" mammalian mitochondria-shaping proteins. The figure depicts the individual domains identified in the core components of the pathway regulating mammalian mitochondrial shape. Note that the regulators highlighted in the text are not represented for the sake of clarity.

common cause of inherited optic neuropathy. OPA1 promotes fusion (18, 58), exists in eight different splice variants (21), and its function appears to be tightly regulated by posttranscriptional mechanisms that include its proteolytic processing. Several proteases have been shown to participate in the cleavage of OPA1. In particular, triple A proteases of the inner mitochondrial membrane cut OPA1 in response to changes in the energetic status of mitochondria (36, 41, 86). This in turn reduces the ability of OPA1 to participate actively in the process of organellar fusion, providing a mechanism to translate mitochondrial dysfunction into changes in morphology of the organelle. The mitochondrial rhomboid protease PARL is involved in the production of a functionally relevant, albeit minor, intermembrane space form of OPA1 (19). This form is required in the regulation of the shape of the cristae and in the protection from apoptotic insults, through the regulation of the so-called cristae-remodelling pathway (19, 32, 82). Conversely, the rhomboid protease does not affect the pro-fusion activity of OPA1, but impinges on its ability to regulate the shape of the cristae, thereby further differentiating the function of this inner-membrane dynamin-related protein (19). It remains to be elucidated whether the action of PARL is somehow in concert with that of the other proteases, like paraplegin and YmeL1, that sense mitochondrial dysfunction to modulate coordinately the multiple functions of OPA1.

LETM1 is an inner-membrane protein, deleted in Wolff-Hirschhorn syndrome, a complex genetic condition, homologue to the yeast regulator of mitochondrial morphology Mdm38p (24). Its ablation results in mitochondrial fragmentation (25, 39). However, it appears that LETM1 does not impinge on the core mechanism of mitochondrial morphology, because its downregulation cannot be complemented by inhibition of the fission machinery (25). Work performed in yeast suggests that Letm1/Mdm38p is involved in the regulation of mitochondrial K^+/H^+ exchange, indicating possible crosstalk between ion homeostasis and regulation of mitochondrial shape (65).

The mechanism of mitochondrial fusion has been closely scrutinized during recent years, especially in yeast. It appears that fusion of the outer mitochondrial membrane requires high levels of GTP, but not mitochondrial membrane potential, whereas fusion of the inner membrane does (59). In mammalian cells, MFN1 docks two juxtaposed mitochondria via its second coiled coil domain to promote fusion (53). The role of the two Mfns in fusion seems to be different. MFN1 has higher GTPase activity and induces fusion more efficiently than MFN2 (40). Furthermore, OPA1 requires MFN1 to mediate fusion, whereas MFN2 functions independent of OPA1 (18).

The list of proteins involved in the control of mitochondrial fusion recently included a phospholipase, PLD, associated with the outer membrane, where it hydrolyzes cardiolipin to generate phosphatidic acid, a lipid involved in SNARE-mediated vesicular fusion (16). Thus, PLD could be the first common component between SNARE-mediated vesicle and mitochondrial fusion. It is conceivable that relative expression levels of these players dictate mitochondrial morphology in different cell types and during development. Along this line, during differentiation from embryonic stem cells into cardiomyocytes, the remarkable changes in mitochondrial ultrastructure and reticular organization are accompanied by the reduction in the levels of OPA1 and by the upregulation of MFN2. This is likely coordinated with the metabolic switch from glycolytic to oxidative (17); the repression of MFN2 in myofibers reduces myocyte respiration, further supporting the liaison between this protein and the mitochondrial oxidative metabolism (4). Furthermore, the conditional deletion of MFN1 and 2 shows that individual cell types are exquisitely sensitive to the ablation of a single protein of the two (14) and that the defect can be complemented by the overexpression of the other; however, it is still to be clarified whether the two MFNs are completely interchangeable in terms of mitochondrial fusion as well as of other cellular functions.

Pro-fission mitochondria-shaping proteins

In mammalian cells, mitochondrial division is regulated by DRP1 and FIS1 (42, 84). The large GTPase DRP1 is a cytosolic dynamin-related protein. Its inhibition or its downregulation results in a highly interconnected mitochondrial network (85). The same phenotype is caused by downregulation of hFIS1 (42). FIS1 is a 16-kDa integral protein of the outer mitochondrial membrane, containing a single transmembrane domain and a tetratricopeptide repeat (TPR, involved in protein–protein interaction) domain facing the cytosol (61). Crystals of human FIS1 revealed that the TPR domain is arranged to expose a hydrophobic cleft on one side of the molecule, suggesting the existence of hydrophobic interactions with DRP1 or with a yet-unidentified adaptor during mitochondrial fission. Some evidence suggests that FIS1 is the receptor on the outer membrane for DRP1, *via* its TPR. DRP1 is recruited to mitochondria, and constriction of the membranes takes place by direct or indirect

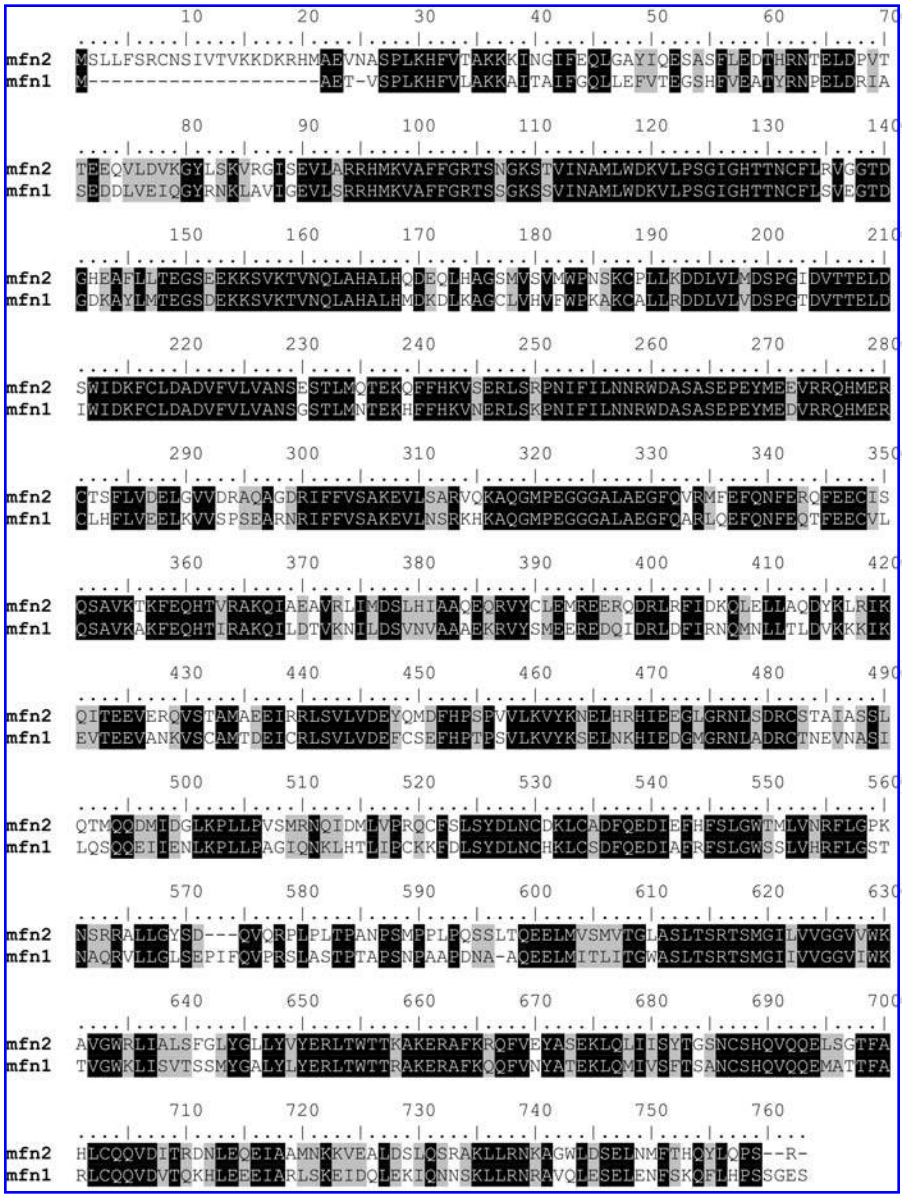

FIG 3. Clustal W alignment of mouse MFN1 and 2. Amino acids shaded in gray are similar, whereas those shaded in black are identical. Note that the N-termini and the areas around aa. 360 and 550 display the highest divergence.

interaction with hFIS1 (97). Levels of FIS1 and DRP1 and therefore mitochondrial fission are proteolytically controlled by the opposing action of sumoylation and ubiquitination (38, 62, 96).

In addition to these two players, endophilin B1, a member of the endophilin family of fatty acid acyl transferases that participate in endocytosis, has been shown to play a role in mitochondrial fission (44). During endocytosis, endophilin 1 builds complexes with dynamin I, the dynamin responsible for the severing of the neck of the nascent endocytic vesicle, and provides the required lipid modification (80). The mechanism by which endophilin B1 regulates mitochondrial shape is unclear, as it can be mediated either by a direct effect on membrane curvature, or by its putative acyl-transferase activity (44).

More recently, ganglioside-differentiation–associated protein 1 (GDAP1), a protein upregulated during cholinergic differentiation of a mouse neuroblastoma cell line induced by ganglioside, was identified as a mediator of mitochondrial fission, although its mechanism of action is completely unknown (64).

GENETIC DISEASES OF MITOCHONDRIA-SHAPING PROTEINS

The crucial role of mitochondria-shaping proteins in the regulation of cell physiology is substantiated by a number of diseases caused by mutations in genes coding for the pro-fusion OPA1, MFN2, and the pro-fission GDAP1, as well as LETM1, whose function in regulation of mitochondrial shape is perhaps secondary. Finally, it was very recently shown that inactivating mutations in DRP1 cause a lethal metabolic syndrome. An overview of the genetic diseases caused by mutations in mitochondria-shaping proteins is presented in Table 1.

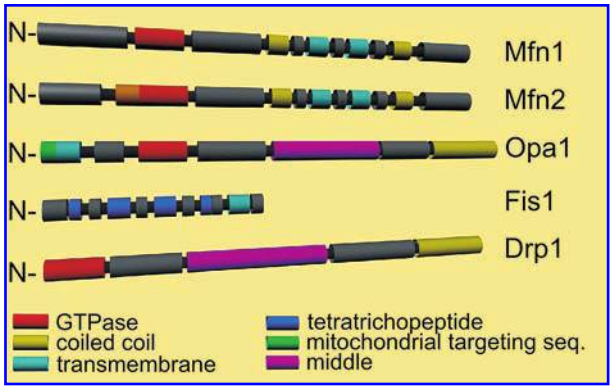

FIG. 4. Topology and functional domains of MFN2. The figure depicts MFN2 in the context of the outer mitochondrial membrane and shows functional domains of the protein in different colors. Gray, p21 Ras–binding domain; purple, GTPase domain; yellow, transmembrane domains. Note that the p21 Ras binding domain is expanded for the sake of clarity.

DRP1-dependent lethal metabolic syndrome

A case of a newborn that rapidly developed a condition of severe metabolic impairment was recently reported, with feeding problems, mild dysmorphia, truncal hypotonia, lack of response to light stimulation, optic atrophy, and abnormal gyral pattern in both frontal lobes that extended to the perisylvian areas and was associated with dysmyelination. Genetic analysis revealed a missense mutation in the central domain of DRP1, mapping to a conserved amino acid. DRP1 participates not only in the fission of mitochondria, but also in the regulation of the shape of other organelles, such as peroxisomes (81). Accordingly, cells from the patient displayed severe defects in the morphology of both organelles. At this point, it is unclear to what extent the clinical phenotype reflects defects in mitochondrial or in peroxisomal function. Clearly, the severity of the clinical picture suggests that both components are involved in the pathogenesis of the disease, but we probably need mouse models to dissect the relative participation of mitochondrial and peroxisomal fission.

Dominant optic atrophy

Mutations in OPA1 are associated with autosomal dominant optic atrophy (ADOA), also known as type I Kjer disease, affecting mainly retinal ganglion cells and causing progressive blindness because of the loss of these neurons (2, 22). Most mutations associated with ADOA cluster in the GTPase and in the coiled coil domain of OPA1 (28, 67). The pathogenesis of the disease results probably from haploinsufficiency. In agreement with this, downregulation of OPA1 by short interfering RNA leads to mitochondrial fragmentation, dissipation of the mitochondrial membrane potential, disorganization of the mitochondrial cristae, decrease in cell respiration, and apoptosis (18, 32, 37, 66) Moreover, two mouse models carrying a truncated OPA1 protein showed signs of retinal ganglion cell loss in the heterozygous population (1, 20). Indeed, pathogenic mutations cause mitochondrial fragmentation and do not protect cells from apoptosis (19, 32). Conversely, overexpression of OPA1 induces mitochondrial elongation; however, it is unclear whether the pro-fusion activity is required to protect from cell death. We therefore wished to verify whether OPA1 acted on the morphology of the inner mitochondrial membrane or of the mitochondrial network. By using a combination of genetics, imaging, electron tomography, and biochemical assays, we demonstrated that OPA1 influences apoptosis independent of its effect on mitochondrial fusion. High levels of wt, but not mutated OPA1 delay apoptosis induced by a set of intrinsic stimuli, by preventing mobilization of cytochrome *c* from the cristae stores and remodelling of the cristae, as visualized by electron tomography. In the search for a molecular mechanism

TABLE 1. THE GENETIC DISEASES OF MITOCHONDRIA-SHAPING PROTEINS

Disease	*Major phenotype*	*Gene affected*	*Mitochondrial morphology*	*Involvement of other organelles*
Dominant optic atrophy	Loss of retinal ganglion cells	OPA1	Probably fragmented	Not known
Charcot Marie Tooth IIA	Loss of sensorimotor axons	MFN2	Probably fragmented	Yes, endoplasmic reticulum
Charcot Marie Tooth IVB	Loss of sensorimotor axons	GDAP	Probably fragmented	Not known
Wolf-Hirschhorn syndrome	seizures	LETM1	Probably fragmented	Not known
Lethal metabolic syndrome	Metabolic and CNS problems	DRP1	elongated	Yes, peroxisomes

Major pathological, genetic and mitochondrial features of genetic diseases associated with mutations in genes coding for mitochondria-shaping proteins are shown. See text for more detail.

by which OPA1 could control the structure of the cristae, we showed that OPA1 is retrieved in high-molecular-weight complexes and that chemical crosslinking can highlight at least an oligomer. This contains both the integral inner membrane and the soluble form of OPA1, as substantiated by experiments of reconstitution with tagged versions of the two OPA1 forms. This oligomer could in principle function as a molecular staple that participates in keeping the tubular junction of the cristae narrow. In line with this hypothesis, mechanical swelling of mitochondria leads to the loss of this oligomer; furthermore, OPA1 oligomers are disrupted early during cristae remodelling and before the release of the cristae stores of cytochrome *c* (32). PARL and OPA1 are genetically positioned in this same pathway regulating cytochrome *c* release, with PARL upstream of OPA1. PARL participates in the generation of a soluble form of OPA1 that is required to control the cristae remodelling pathway, as mentioned earlier. The importance of this pathway was substantiated by the analysis of a mouse knockout of *Parl*, which has an extremely shortened life span because of progressive cachexia and sustained apoptosis *in situ* and *ex vivo* in cells from all the tissue affected. Mouse embryonic fibroblasts (MEFs) lacking *Parl* recapitulate the increased apoptosis observed in the animal. The molecular analysis of the phenotype revealed that PARL controls the kinetics of cytochrome *c* release from mitochondria, by impinging on the cristae-remodelling pathway (19). This work provided the founding base to test (a) the molecular regulation of cristae structure that probably impinges on the high-molecular-weight complex of OPA1; and (b) the pathogenesis of dominant optic atrophy, by dissecting the relative role of the mutants of OPA1 in mitochondrial fusion and cristae remodelling in retinal ganglion cells. For the first time, we described a role for the mitochondrial rhomboid protease PARL in apoptosis. Finally, it opens the possibility that deranged OPA1 processing by PARL is relevant in the pathogenesis of dominant optic atrophy.

Although it remains to be clarified whether ADOA is caused by the impairment of the pro-fusion or the antiapoptotic role of OPA1, this represents the first evidence that mitochondria-shaping proteins can live more than one life. They fulfil their original biologic task by regulating the shape of the organelle, but they are also essential regulators of other genetically distinct pathways.

Wolf–Hirschhorn syndrome

The Wolf–Hirschhorn syndrome (WHS) has an incidence of 1/50,000 live births and is characterized by severe pre- and postnatal growth retardation, impairment of muscular tone, severe mental retardation, developmental delay with microcephaly, a characteristic facial appearance, and midline fusion defects . Seizures occur in practically all the cases and are one of the major characteristics of the full WHS phenotype (99). Epilepsy in WHS usually starts during the first year of life and is a frequent cause of short survival. It has been suggested that the electroclinical similarities between WHS and Angelman syndrome, in which γ-aminobutyric acid $(GABA)_A$-receptor genes are involved, might represent a characteristic type of epilepsy (6). In the WHS-critical region, no GABA-receptor gene could be identified, indicating that no common pathogenetic mechanism involves the GABA signaling pathway (26). WHS is caused by the partial deletion of the short arm of one chromosome 4, involving chromosome region 4p16.3. The WHS is thought to be a contiguous gene syndrome with an unknown number of genes contributing to the phenotype. Recently, a novel gene (*LETM1*) was cloned (26), located <80 kb distal to the critical region deleted in WHS patients. Remarkably, *LETM1* is deleted in all WHS patients with the full phenotype, including the occurrence of seizures, suggesting a role for its haploinsufficiency in the pathogenesis of seizures (99). *LETM1* consists of 14 exons, and Northern blot analysis revealed ubiquitous expression. LETM1 encodes a putative 83.4-kDa membrane-bound protein, which is a member of the superfamily of EF-hand Ca^{2+}-binding proteins and contains a leucine zipper and several α-helical regions with the ability to form coiled coils, suggesting a function in Ca^{2+} signaling or homeostasis. Further functional characterization by the group revealed that LETM1 is evolutionary conserved throughout the eukaryotic kingdom (79). The sequence identity ranges from 31% in *Saccharomyces cerevisiae* to 41% in *D. melanogaster* and 83% in mouse, suggesting that these proteins may be encoded by homologous genes. Several functional domains, including EF-hand motifs, a highly conserved PKC/CKII phosphorylation site, a transmembrane domain, coiled-coil regions, and a leucine zipper in some of the proteins, are conserved in these proteins. In addition, a highly conserved possible SAP domain C-terminal to the transmembrane domain could be detected. Interestingly, the SAP-like region is present in all species, again at an invariant position, 138 to 145 amino acids C-terminal to the transmembrane domain. The SAP domain or SAF-box is a putative bihelical DNA-binding domain that is predicted to bind to scaffold-attachment regions (SARs) with high specificity and may therefore be involved in chromosomal organization.

Interestingly, LETM1 exhibits homology to the mitochondrial yeast protein Mdm38p, and it is indeed a mitochondrial protein (25, 39, 79). MDM38, which is a homologue of LETM1, is proposed to be involved in organelle morphology and to participate in K^+/H^+ exchange across the inner mitochondrial membrane (24, 65), or in export of protein precursors from the matrix to the inner mitochondrial membrane (30). The analysis of Letm1 function in higher organisms shows that it critically regulates mitochondrial morphology: its downregulation in mammalian cells as well as in *C. elegans* causes mitochondrial fragmentation (25, 39), although this process is not reverted by the genetic inhibition of the fission machinery (25). This raises the possibility that the fragmentation is a secondary consequence of deranged mitochondrial function, thus leaving open the possibility that Letm1 participates in correct assembly of the respiratory chain, or in the regulation of mitochondrial ion homeostasis. Interestingly, viability is the cellular function that is most sensitive to this change in mitochondrial shape that triggers a nonapoptotic death cascade (25), without overt or latent mitochondrial dysfunction. This suggests that cells critically rely on mitochondrial shape, not only for the apoptotic cascade, but also to maintain viability and probably ion homeostasis.

Charcot-Marie-Tooth (CMT) type 4A

CMT is one of the most commonly inherited disorders in humans, with an estimated prevalence of one in 2,500 indi-

viduals. CMT is a peripheral sensorimotor neuropathy characterized by distal weakness of the lower limbs, sensory loss, decreased reflexes, and foot deformities. Other symptoms include cranial nerve involvement, scoliosis, vocal cord paresis, and glaucoma. CMT neuropathies can be divided into four main groups, types 1 to 4. In CMT1, -3, and -4, nerve-conduction velocities are considerably reduced. In CMT2, nerve-conduction velocities are normal, but conduction amplitudes are decreased, because of the degeneration of nerve fibers (102). CMT1, CMT3, and CMT4 are demyelinating forms associated with segmental de- and remyelination, whereas CMT2, the axonal form, shows axonal degeneration without demyelination (9, 89). The subtype CMT4A is the most frequent recessive form of CMT and has been associated to mutations in GDAP1. Remarkably, the 20 mutations in GDAP associated with CMT4A can cause axonal, demyelinating, or intermediate forms (23), without any correlation between the mutations and the clinical phenotype. GDAP1 displays a certain degree of homology with glutathione-S-transferase (GST) and is expressed in different regions of the central nervous system, including the cortex, cerebellum, thalamus, olfactory bulb, and spinal cord, as well as in sciatic nerves and dorsal root ganglia. GDAP1 is a mitochondrial outer membrane protein that promotes mitochondrial fission, and clinically relevant mutations impair its pro-fission activity (64). How these mutations result in such diverse clinical phenotypes is unclear. A tempting hypothesis is that GDAP1 is involved in the detoxification of peroxidized mitochondrial lipids *via* its GST activity. A very low degree of GST activity was reported at physiologic pH for a truncated version of the protein lacking the transmembrane domains required for its mitochondrial localization (70). Although this could in principle suggest that the pro-fission activity of GDAP1 is unrelated to its participation in detoxifying reactions at the surface of mitochondria, it should be noted that the two transmembrane domains might be required to activate the GST activity of the protein. Furthermore, pharmacologic depletion of mitochondrial glutathione (GSH) pools results in the formation of an elongated mitochondrial reticulum, suggesting a link between GSH, redox status, and mitochondrial morphology (10).

Charcot-Marie-Tooth type 2A

In 2004, Zuchner *et al*. (133) mapped the mutations responsible for CMT2A, and identified *MFN2* as the gene responsible for this disorder. In CMT2A, the loss of sensory and motor axons eventually results in the degeneration of the neurons themselves. Mutations in *MFN2* account for ~20% of CMT2 cases, making this the most prevalent axonal form of CMT. Most *MFN2* mutations in CMT2A cluster within the GTPase and the RAS-binding domains and are missense mutations (49, 54, 101). Recently, a *de novo* truncation mutation in *MFN2* was associated with CMT2 and optic atrophy (also known as hereditary motor and sensory neuropathy VI, HMSN VI) (100). It is intriguing that both mutations in *MFN2* and in *OPA1* can cause optic atrophy, suggesting that the functional impairment of these two molecules eventually converges on a common pathway, causing optic nerve degeneration. The mechanism by which mutations in *MFN2* cause CMT2A is unknown, although it was recently suggested that it could be the consequence of reduced axonal movement of mitochondria.

The first hint of a role for mitofusins in mitochondrial movement was given by the analysis of mouse embryonic fibroblasts (MEFs) lacking *Mfn1* or *Mfn2*. In wild-type MEFs, mitochondria move back and forth along the long axis of the cell on radial tracks. In *Mfn1*−/− cells, mitochondria display a disorganized, "brownian-like" movement. Conversely, *Mfn2*−/− cells contained both tubular mitochondria moving in an organized way as in wt cells and spherical mitochondria that presented uncoordinated motion (13). In addition, the expression of pathogenic mutants of MFN2 in neurons significantly reduces transport of mitochondria from the cell body to the distal segments of long motor and sensory axons (5). A conditional mouse model of MFN2 ablation also showed greatly reduced mitochondrial movement and accumulation in axons or Purkinje cells of the cerebellum (14). Thus, MFN2 may play a regulatory role in keeping the interaction between mitochondria and the organelle transport machinery. Given that most mutations cluster in the GTPase domain, the liaison should depend on the ability of MFN2 to hydrolyze GTP, suggesting that MFN2 could be similar to Miro1 and 2, two other outer membrane GTPases that act as mitochondrial receptors in *Drosophila* for Milton, the linker between mitochondria and the microtubular cargoes (87).

MFN2 AND NON-NEURODEGENERATIVE DISEASES: LIGHT ON A MULTIFACETED BIOLOGY?

MFN2 has been associated with a number of different pathologic conditions, ranging from neurodegeneration to diabetes. As described earlier, MFN2 is an outer mitochondrial membrane protein that participates in mitochondrial fusion. However, it is believed that its role extends beyond fusion and that it is functionally divergent from its homologue, MFN1.

MFN1 and MFN2 are both transmembrane GTPases of the outer mitochondrial membrane that display high homology (81%) and similar topology (76, 78). MFN1 and MFN2 are expressed at low levels in many tissues. MFN1 levels are higher in heart and testis; those of MFN2 are increased in heart, skeletal muscle, tongue, and brain (27, 77). The MFNs possess a GTPase and a coiled-coil domain located at the N-terminus of the proteins, exposed to the cytosol. Two TM regions form a U-shaped membrane anchor, ending in a cytosolic, C-terminal coiled-coil motif (53, 76). MFN1 on opposing mitochondrial membranes can bind in trans to bridge organelles, maintaining a distance of 95 Å between the two membranes (53). Whether this ability is shared by MFN2 is not yet clear. Conversely, MFN2 possesses an N-terminal RAS-binding domain that is not found in MFN1 (15), suggesting a role in other processes than membrane fusion.

Differences between mitofusins in promotion of mitochondrial fusion

The differential role played by the two mitofusins during mitochondrial fusion was first demonstrated by directly measur-

ing mitochondrial fusion rates in *Mfn1*−/− and *Mfn2*−/− cells. These experiments substantiated that cells containing only MFN1 retain more fusion activity than those that contain only MFN2 (13). Other experimental evidence strengthened this experimental observation: MFN1 mediates GTP-dependent tethering of mitochondria more efficiently than MFN2, and its GTPase activity is higher than that of MFN2, even if MFN2 has a greater affinity for GTP (40, 63). Finally, MFN1 but not MFN2 is essential for OPA1-mediated mitochondrial fusion (18). Extending these cell biologic observations, genetic ablation of the two genes in the mouse does not result in the same phenotype: *Mfn1*−/− mice die in midgestation, whereas *Mfn2*−/− embryos display deficient placentation. Overall, these observations suggest that if MFNs can obviously share a common role in mitochondrial fusion; they appear probably to regulate this process in different manners and to have additional functions. In particular, increasing experimental evidence is mounting on the role of MFN2 in different pathologic and physiologic conditions, as we now discuss in more detail.

MFN2 and type 2 diabetes mellitus

In vivo and *in vitro* models show that physiologic and pathophysiologic conditions characterized by altered glucose oxidation, such as diabetes, obesity, insulin resistance, exercise, and weight loss, lead to changes in MFN2 expression (4, 71). Conditions associated with defective mitochondrial oxidative phosphorylation, such as obesity and type 2 diabetes mellitus, are associated with reduced MFN2 gene expression (3, 4); conversely, exercise and weight loss are related to increases in MFN2 expression (11, 60). After acute physical exercise, MFN2 synthesis is activated by the transcription factors PGC-1α and ERRα (11, 33). Another striking observation is that mitochondrial volume is reduced by 35% in skeletal muscle from obese and type 2 diabetes patients (48). Insulin in diabetes patients recovers mitochondrial structural changes (92); furthermore, insulin upregulates MFN2 levels and induces mitochondrial biogenesis, probably by blocking the MEK-dependent cascade and by activating the PI3K signaling pathway (69).

These results open the possibility that MFN2 has an additional role in regulating mitochondrial metabolism, although it is unclear whether this outer mitochondrial membrane protein fulfils this role by influencing signaling cascades, or by a direct mitochondrial effect. The former hypothesis is substantiated by the potential role of MFN2 in regulating Ras-dependent signaling that emerged in analyzing its role in vascular proliferation.

MFN2 and vascular proliferative diseases

MFN2 expression levels have been linked to vascular proliferative diseases: in hyperproliferating vascular smooth muscle cells, *MFN2* expression is downregulated (15). The overexpression of MFN2 in these cells physically sequesters Ras, thereby inhibiting the downstream Ras signaling pathway, inactivating the ERK1/2 cascade, and eventually arresting the cell cycle in the G_0/G_1 phase. This action of MFN2 is independent of its mitochondrial localization and is also observed *in vivo* in response to arterial injury and in animal models of atherosclerosis, suggesting that MFN2 could be a main contributor in proliferative cardiovascular disorders and suggesting a possible contribution for MFN2 in other disorders resulting from altered cell proliferation, such as cancer. Moreover, it provides a rationale for the effect of MFN2 on mitochondrial metabolism, *via* an unexpected signaling loop that is interrupted at the surface of mitochondria where Ras is sequestered.

The potential role of MFN2 in neoplastic transformation is furthermore suggested by the recent finding of interplay between MFN2 and the proapoptotic Bcl-2 family member BAX.

MFN2, BAX, and fusion

Changes in mitochondrial shape are associated with the activation of the mitochondrial pathway of apoptosis. BAX translocation from cytosol to mitochondria is followed by extensive mitochondrial fragmentation. This is mediated by DRP1 translocation from the cytosol to mitochondria, where DRP1 localizes and mediates mitochondrial fission at foci of BAX accumulation and occurs before caspase activation (29, 44, 57). Inhibition of mitochondrial fission delays activation of downstream caspases and cell death. Moreover, elegant single-cell studies by Karbowski *et al*. (43) demonstrated that mitochondrial fragmentation is also caused by the inhibition of MFN1-dependent mitochondrial fusion.

What is the role of MFN2 in apoptosis? Overexpression of both MFN1 and 2 can delay apoptosis by a number of intrinsic stimuli (88). Furthermore, constitutively active MFN2 prevents BAX translocation on mitochondria, further strengthening the interplay between MFN2 and BAX (63). MFN2, but not MFN1, is sequestered by BAX at specific foci on mitochondria in nonapoptotic conditions (47). In cells lacking BAX, a high-molecular-weight complex of MFN2, probably involved in mitochondrial fusion, is disrupted. After induction of apoptosis, activated BAX probably adopts a conformation that is unable to interact with MFN2, leading to relocalization of MFN2 on the outer mitochondrial membrane. At the same time, BAX activation promotes DRP1 translocation to mitochondria to induce fission (47). In conclusion, MFN2 appears to regulate activation of the multidomain proapoptotics that regulate efflux of cytochrome *c* from mitochondria. Reciprocally, BAX is required for the correct assembly of MFN2 oligomers, substantiating cross-talk between mitochondria-shaping proteins and regulators of apoptosis, independent of the induction of cell death.

MITOCHONDRIA-SHAPING PROTEINS AND ROS PRODUCTION: AN OPEN QUESTION

As we saw earlier, the link between ROS production and mitochondrial morphology is still unclear. The notion that mitochondria are among the most prominent cellular sources of ROS is well accepted, and a discussion on this topic is beyond the scope of this review. Moreover, mitochondria are also crucial targets of ROS that can modulate the function of this organelle under normal and pathologic conditions. Therefore, when considering the relation between mitochondrial shape and ROS, one is urged to analyze it in both directions: what happens to

mitochondrial morphology under conditions of increased ROS production? Can changes in mitochondrial shape influence production of ROS by mitochondria or by other organelles?

Although it is easier to imagine that changing the rate of ROS production would somehow affect mitochondrial shape, we recently learned that mitochondrial fission mediated by DRP1 is a necessary component for high glucose–induced respiration increase and ROS overproduction (98). How this occurs is unknown. A tempting speculation would link mitochondrial fission to intramitochondrial redistribution of cytochrome *c* and p66shc, the mitochondrial product of the pro-aging gene *shc*. Ablation of p66shc greatly enhances life span of the mouse concomitant with a reduced production of ROS by this organelle. p66shc serves as alternative electron acceptor for cytochrome *c*, eventually leading to the production of H_2O_2 (35). The action of p66 requires that it is proximal to cytochrome *c*, which is mostly sequestered in the cristae. However, DRP1-dependent mitochondrial fission has been linked to .cytochrome *c* redistribution and cristae remodelling (34), a process normally observed during apoptosis and regulated by OPA1 in cooperation with the rhomboid protease PARL (19, 32, 82). Redistribution of cytochrome *c* would then be crucial to co-compartmentalize p66shc and its electron donor, allowing production of H_2O_2.

The analysis of the relation between mitochondrial morphology and ROS overload leads to some surprises. In a set of pioneering studies, Wakabayashi and Karbowski (45, 46) identified the production of "megamitochondria" as early markers of ROS accumulation. The morphology as well as the ultrastructure of these mitochondria is strikingly similar to that of the organelles in cells overexpressing pro-fusion mitochondria-shaping proteins such as MFN2 (76, 78). Formation of the megamitochondria indeed requires ROS, but is also dependent on protein synthesis (90). This suggests that upregulation of pro-fusion proteins like MFN2 could be a crucial event in the mitochondrial response to oxidative stress. In conclusion, mitochondria-shaping proteins seem to play a role in the mitochondrial generation of and in response to ROS. In this context, MFN2 could emerge as a regulator of the mitochondrial response, perhaps by influencing mitochondrial metabolism, or by affecting extramitochondrial signaling pathways that are crucial in the cellular response to ROS, such as the ERK-JNK pathway (91).

Another link between proteins that regulate mitochondrial shape and ROS production comes from their role outside mitochondria. DRP1 and FIS1, the mitochondrial fission proteins, are also localized on peroxisomes, where they regulate the morphology of this organelle and therefore its proliferation (50–52, 56). It is therefore conceivable that in response to ROS, cells take a concerted action on both mitochondrial and peroxisomal morphology to compensate for the overproduction of the toxic radicals. In this respect, sharing the components of the machinery that regulates the shape of these two organelles would make cells' life easier.

OTHER EXTRAMITOCHONDRIAL ROLES OF MITOCHONDRIA-SHAPING PROTEINS

Peroxisomes are not the sole nonmitochondrial organelle whose shape is influenced by mitochondria-shaping proteins. For example, DRP1 also affects the shape of the endoplasmic reticulum (72). The functional consequences of DRP1 localization at the ER are not yet known. It is possible to speculate that division of mitochondria and ER should proceed in sync to preserve the areas of juxtaposition between the two organelles required for Ca^{2+} and lipid transfer (75).

Similarly, we recently showed that MFN2 can regulate ER morphology and ER-to-mitochondria interaction (Martins de Brito and Scorrano, unpublished data). The ER is a continuous membrane-bound organelle present in all eukaryotic cells (7). The ER is composed of at least three distinct subcompartments: the nuclear envelope, the ribosome-bound rough ER, and the ribosome-free smooth ER (95). The smooth and rough ER form a network of interconnected tubules that extend throughout the cell cytoplasm and is called peripheral ER. Most proteins are shared between RER and SER, but several proteins involved in translocation or processing of newly synthesized proteins are enriched in the RER (95). The RER is present in all cell types, and this can be explained by the fact that protein translocation is essential for all eukaryotic cells. Another type of SER that is present in all cell types is the transitional ER (68). This structure is involved in packaging proteins for transport from the ER to the Golgi and is enriched in proteins required for this process (95). The peripheral ER is composed of sheet-like cisternae and a polygonal array of tubules connected by three-way junctions. ER tubules are long cylindrical units with high membrane curvature in cross section. The thickness of a sheet and the diameter of a tubule is typically 60–100 nm (95). This conserved regularity of the domains suggests that both sheets and tubules are being shaped actively. However, very little is known about the regulation of ER morphology. Motor and nonmotor proteins that maintain the interaction between cytoskeleton and the ER seem to play an important role in stabilizing the morphology of this organelle. Besides cytoskeleton-interacting proteins, recent work by Rapoport (83, 94) shows a role for the membrane-bending protein Rtn4a. This protein adopts a hairpin topology on the cytoplasmic leaflet of the ER membrane. The unusual topology of Rtn4a produces a wedge-shaped intramembrane domain that causes bending of the phospholipid bilayer, promoting the high membrane curvature of the ER network.

Recently we showed by a loss-of-function approach that MFN2 also has a role in regulating ER morphology. We found that MFN2 localized not only on mitochondria, but it also was highly enriched in the mitochondria-associated membrane fraction and present (albeit to a lesser extent) at the ER. ER localization of MFN2 proved necessary to maintain the reticular and interconnected morphology of this organelle. Further, we found that from its ER position, MFN2 could control ER-to-mitochondria interaction, by forming complexes with MFN1 or MFN2 on mitochondria that can be stabilized by chemical crosslinking. In conclusion, we found that whereas mitochondrial MFN2 regulates shape of this organelle, ER-associated MFN2 is required for ER morphology and tethering to mitochondria. As a functional consequence, during physiologic, inositol trisphosphate–mediated Ca^{2+} signaling, mitochondria in *Mfn2-/-* cells uptake less Ca^{2+} and more slowly than their wt counterparts, proving that the microdomains of high Ca^{2+} generated by the juxtaposition of the two organelles are required for efficient mitochondrial Ca^{2+} uptake (74, 75). The unex-

pected role for MFN2 at the ER and in regulating juxtaposition with mitochondria opens the possibility that mitochondria-shaping proteins affect signaling not only by changing the shape of their organelle of origin, but also by affecting other cellular structures.

CONCLUSIONS

Proteins involved in the control of mitochondrial shape participate in multiple cellular pathways, ranging from Ca^{2+} to regulation of apoptosis, to production of ROS, and even to the control of life span. We expect that the list of the signaling cascades influenced by mitochondria-shaping proteins is far from complete. As research progresses, we will discover other "moonlighting" jobs of mitochondria-shaping proteins that are likely to place our favorite organelle in the (center) stage of many other signaling pathways.

ACKNOWLEDGMENTS

We thank C. Frezza of our laboratory for the artwork. O.M.d.B. is the recipient of a "Bolsa de Doutoramento" of the "Fundação para a Ciência e Tecnologia," Portugal. L.S. is Assistant Telethon Scientist of the Dulbecco-Telethon Institute and an EMBO Young Investigator. Research in his laboratory is supported by Telethon Italy, Compagnia di San Paolo Italy, United Mitochondrial Disease Fund USA, MDA USA, AIRC Italy, and Fondazione Mariani ONLUS-Italy.

ABBREVIATIONS

ADOA, Autosomal dominant optic atrophy; AIF, apoptosis-inducing factor; DRP1, dynamin-related protein 1; CMT, Charcot-Marie-Tooth; ER, endoplasmic reticulum; GDAP1, ganglioside-induced differentiation-associated protein 1; GSH, glutathione; GST, gluthatione-S-transferase; HMSN, hereditary motor and sensory neuropathy; IMM, inner mitochondrial membrane; IMS, intermembrane space; Mfn, mitofusin; OPA1, optic atrophy 1; Parl, presenilin-associated rhomboid like; ROS, reactive oxygen species; TCA, tricarboxylic acid; TM, transmembrane.

REFERENCES

1. 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, and Wissinger B. A splice site mutation in the murine Opa1 gene features pathology of autosomal dominant optic atrophy. *Brain* 130: 1029–1042, 2007.
2. Alexander C, Votruba M, Pesch UE, Thiselton DL, Mayer S, Moore A, Rodriguez M, Kellner U, Leo-Kottler B, Auburger G, Bhattacharya SS, and Wissinger B. OPA1, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28. *Nat Genet* 26: 211–215, 2000.
3. Bach D, Naon D, Pich S, Soriano FX, Vega N, Rieusset J, Laville M, Guillet C, Boirie Y, Wallberg-Henriksson H, Manco M, Calvani M, Castagneto M, Palacin M, Mingrone G, Zierath JR, Vidal H, and Zorzano A. Expression of Mfn2, the Charcot-Marie-Tooth neuropathy type 2A gene, in human skeletal muscle: effects of type 2 diabetes, obesity, weight loss, and the regulatory role of tumor necrosis factor alpha and interleukin-6. *Diabetes* 54: 2685–2693, 2005.
4. Bach D, Pich S, Soriano FX, Vega N, Baumgartner B, Oriola J, Daugaard JR, Lloberas J, Camps M, Zierath JR, Rabasa-Lhoret R, Wallberg-Henriksson H, Laville M, Palacin M, Vidal H, Rivera F, Brand M, and Zorzano A. Mitofusin-2 determines mitochondrial network architecture and mitochondrial metabolism: a novel regulatory mechanism altered in obesity. *J Biol Chem* 278: 17190–17197, 2003.
5. Baloh RH, Schmidt RE, Pestronk A, and Milbrandt J. Altered axonal mitochondrial transport in the pathogenesis of Charcot-Marie-Tooth disease from mitofusin 2 mutations. *J Neurosci* 27: 422–430, 2007.
6. Battaglia D, Zampino G, Zollino M, Mariotti P, Acquafondata C, Lettori D, Pane M, Vasta I, Neri G, Dravet C, and Guzzetta F. Electroclinical patterns and evolution of epilepsy in the 4p- syndrome. *Epilepsia* 44: 1183–1190, 2003.
7. Baumann O and Walz B. Endoplasmic reticulum of animal cells and its organization into structural and functional domains. *Int Rev Cytol* 205: 149–214, 2001.
8. Bereiter-Hahn J and Voth M. Dynamics of mitochondria in living cells: shape changes, dislocations, fusion, and fission of mitochondria. *Microsc Res Tech* 27: 198–219, 1994.
9. Berger P, Young P, and Suter U. Molecular cell biology of Charcot-Marie-Tooth disease. *Neurogenetics* 4: 1–15, 2002.
10. Bowes TJ and Gupta RS. Induction of mitochondrial fusion by cysteine-alkylators ethacrynic acid and N-ethylmaleimide. *J Cell Physiol* 202: 796–804, 2005.
11. Cartoni R, Leger B, Hock MB, Praz M, Crettenand A, Pich S, Ziltener JL, Luthi F, Deriaz O, Zorzano A, Gobelet C, Kralli A, and Russell AP. Mitofusins 1/2 and ERRalpha expression are increased in human skeletal muscle after physical exercise. *J Physiol* 567: 349–358, 2005.
12. Cereghetti GM and Scorrano L. The many shapes of mitochondrial death. *Oncogene* 25: 4717–4724, 2006.
13. Chen H, Detmer SA, Ewald AJ, Griffin EE, Fraser SE, and Chan DC. Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. *J Cell Biol* 160: 189–200, 2003.
14. Chen H, McCaffery JM, and Chan DC. Mitochondrial fusion protects against neurodegeneration in the cerebellum. *Cell* 130: 548–562, 2007.
15. Chen KH, Guo X, Ma D, Guo Y, Li Q, Yang D, Li P, Qiu X, Wen S, Xiao RP, and Tang J. Dysregulation of HSG triggers vascular proliferative disorders. *Nat Cell Biol* 6: 872–883, 2004.
16. Choi SY, Huang P, Jenkins GM, Chan DC, Schiller J, and Frohman MA. A common lipid links Mfn-mediated mitochondrial fusion and SNARE-regulated exocytosis. *Nat Cell Biol* 8: 1255–1262, 2006.
17. Chung S, Dzeja PP, Faustino RS, Perez-Terzic C, Behfar A, and Terzic A. Mitochondrial oxidative metabolism is required for the cardiac differentiation of stem cells. *Nat Clin Pract Cardiovasc Med* 4(suppl 1): S60–S67, 2007.
18. Cipolat S, de Brito OM, Dal Zilio B, and Scorrano L. OPA1 requires mitofusin 1 to promote mitochondrial fusion. *Proc Natl Acad Sci U S A* 101: 15927–15932, 2004.
19. 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, and De Strooper B. Mitochondrial rhomboid PARL regulates cytochrome c release during apoptosis via OPA1-dependent cristae remodeling. *Cell* 126: 163–175, 2006.
20. Davies VJ, Hollins AJ, Piechota MJ, Yip W, Davies JR, White KE, Nicols PP, Boulton ME, and Votruba M. 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, 2007.
21. Delettre C, Griffoin JM, Kaplan J, Dollfus H, Lorenz B, Faivre L, Lenaers G, Belenguer P, and Hamel CP. Mutation spectrum

and splicing variants in the OPA1 gene. *Hum Genet* 109: 584–591, 2001.
22. Delettre C, Lenaers G, Griffoin JM, Gigarel N, Lorenzo C, Belenguer P, Pelloquin L, Grosgeorge J, Turc-Carel C, Perret E, Starie-Dequeker C, Lasquellec L, Arnaud B, Ducommun B, Kaplan J, and Hamel CP. Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. *Nat Genet* 26: 207–210, 2000.
23. Di Maria E, Gulli R, Balestra P, Cassandrini D, Pigullo S, Doria-Lamba L, Bado M, Schenone A, Ajmar F, Mandich P, and Bellone E. A novel mutation of GDAP1 associated with Charcot-Marie-Tooth disease in three Italian families: evidence for a founder effect. *J Neurol Neurosurg Psychiatry* 75: 1495–1498, 2004.
24. Dimmer KS, Fritz S, Fuchs F, Messerschmitt M, Weinbach N, Neupert W, and Westermann B. Genetic basis of mitochondrial function and morphology in *Saccharomyces cerevisiae*. Mol Biol Cell 13: 847–853, 2002.
25. Dimmer KS, Navoni F, Casarin A, Trevisson E, Endele S, Winterpacht A, Salviati L, and Scorrano L. *LETM1*, deleted in Wolf-Hirschhorn syndrome regulates mitochondrial morphology and cellular viability. *Hum Mol Genet* 2007.
26. Endele S, Fuhry M, Pak SJ, Zabel BU, and Winterpacht A. LETM1, a novel gene encoding a putative EF-hand Ca(2+)-binding protein, flanks the Wolf-Hirschhorn syndrome (WHS) critical region and is deleted in most WHS patients. *Genomics* 60: 218–225, 1999.
27. Eura Y, Ishihara N, Yokota S, and Mihara K. Two mitofusin proteins, mammalian homologues of FZO, with distinct functions are both required for mitochondrial fusion. *J Biochem (Tokyo)* 134: 333–344, 2003.
28. Ferre M, Mati-Bonneau P, Tourmen Y, Malthiery Y, and Reynier P. eOPA1: An online database for OPA1 mutations. *Hum Mutat* 25: 423–428, 2005.
29. Frank S, Gaume B, Bergmann-Leitner ES, Leitner WW, Robert EG, Catez F, Smith CL, and Youle RJ. The role of dynamin-related protein 1, a mediator of mitochondrial fission, in apoptosis. *Dev Cell* 1: 515–525, 2001.
30. Frazier AE, Taylor RD, Mick DU, Warscheid B, Stoepel N, Meyer HE, Ryan MT, Guiard B, and Rehling P. Mdm38 interacts with ribosomes and is a component of the mitochondrial protein export machinery. *J Cell Biol* 172: 553–564, 2006.
31. Frey TG and Mannella CA. The internal structure of mitochondria. *Trends Biochem Sci* 25: 319–324, 2000.
32. Frezza C, Cipolat S, Martins dB, Micaroni M, Beznoussenko GV, Rudka T, Bartoli D, Polishuck RS, Danial NN, De Strooper B, and Scorrano L. OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion. *Cell* 126: 177–189, 2006.
33. Garnier A, Fortin D, Zoll J, N'Guessan B, Mettauer B, Lampert E, Veksler V, and Ventura-Clapier R. Coordinated changes in mitochondrial function and biogenesis in healthy and diseased human skeletal muscle. *FASEB J* 19: 43–52, 2005.
34. Germain M, Mathai JP, McBride HM, and Shore GC. Endoplasmic reticulum BIK initiates DRP1-regulated remodelling of mitochondrial cristae during apoptosis. *EMBO J* 24: 1546–1556, 2005.
35. Giorgio M, Migliaccio E, Orsini F, Paolucci D, Moroni M, Contursi C, Pelliccia G, Luzi L, Minucci S, Marcaccio M, Pinton P, Rizzuto R, Bernardi P, Paolucci F, and Pelicci PG. Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis. *Cell* 122: 221–233, 2005.
36. Griparic L, Kanazawa T, and van der Bliek AM. Regulation of the mitochondrial dynamin-like protein Opa1 by proteolytic cleavage. *J Cell Biol* 178: 757–764, 2007.
37. Griparic L, van der Wel NN, Orozco IJ, Peters PJ, and van der Bliek AM. Loss of the intermembrane space protein Mgm1/OPA1 induces swelling and localized constrictions along the lengths of mitochondria. *J Biol Chem* 279: 18792–18798, 2004.
38. Harder Z, Zunino R, and McBride H. Sumo1 conjugates mitochondrial substrates and participates in mitochondrial fission. *Curr Biol* 14: 340–345, 2004.
39. Hasegawa A and van der Bliek AM. Inverse correlation between expression of the Wolfs Hirschhorn candidate gene Letm1 and mitochondrial volume in C. elegans and in mammalian cells. *Hum Mol Genet* 16: 2061–2071, 2007.
40. Ishihara N, Eura Y, and Mihara K. Mitofusin 1 and 2 play distinct roles in mitochondrial fusion reactions via GTPase activity. *J Cell Sci* 117: 6535–6546, 2004.
41. Ishihara N, Fujita Y, Oka T, and Mihara K. Regulation of mitochondrial morphology through proteolytic cleavage of OPA1. *EMBO J* 25: 2966–2977, 2006.
42. James DI, Parone PA, Mattenberger Y, and Martinou JC. hFis1, a novel component of the mammalian mitochondrial fission machinery. *J Biol Chem* 278: 36373–36379, 2003.
43. Karbowski M, Arnoult D, Chen H, Chan DC, Smith CL, and Youle RJ. Quantitation of mitochondrial dynamics by photolabeling of individual organelles shows that mitochondrial fusion is blocked during the Bax activation phase of apoptosis. *J Cell Biol* 164: 493–499, 2004.
44. Karbowski M, Jeong SY, and Youle RJ. Endophilin B1 is required for the maintenance of mitochondrial morphology. *J Cell Biol* 166: 1027–1039, 2004.
45. Karbowski M, Kurono C, Nishizawa Y, Horie Y, Soji T, and Wakabayashi T. Induction of megamitochondria by some chemicals inducing oxidative stress in primary cultured rat hepatocytes. *Biochim Biophys Acta* 1349: 242–250, 1997.
46. Karbowski M, Kurono C, Wozniak M, Ostrowski M, Teranishi M, Nishizawa Y, Usukura J, Soji T, and Wakabayashi T. Free radical-induced megamitochondria formation and apoptosis. *Free Radic Biol Med* 26: 396–409, 1999.
47. Karbowski M, Norris KL, Cleland MM, Jeong SY, and Youle RJ. Role of Bax and Bak in mitochondrial morphogenesis. *Nature* 443: 658–662, 2006.
48. Kelley DE, He J, Menshikova EV, and Ritov VB. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes* 51: 2944–2950, 2002.
49. Kijima K, Numakura C, Izumino H, Umetsu K, Nezu A, Shiiki T, Ogawa M, Ishizaki Y, Kitamura T, Shozawa Y, and Hayasaka K. Mitochondrial GTPase mitofusin 2 mutation in Charcot-Marie-Tooth neuropathy type 2A. *Hum Genet* 116: 23–27, 2005.
50. Koch A, Schneider G, Luers GH, and Schrader M. Peroxisome elongation and constriction but not fission can occur independently of dynamin-like protein 1. *J Cell Sci* 117: 3995–4006, 2004.
51. Koch A, Thiemann M, Grabenbauer M, Yoon Y, McNiven MA, and Schrader M. Dynamin-like protein 1 is involved in peroxisomal fission. *J Biol Chem* 278: 8597–8605, 2003.
52. Koch A, Yoon Y, Bonekamp NA, McNiven MA, and Schrader M. A role for Fis1 in both mitochondrial and peroxisomal fission in mammalian cells. *Mol Biol Cell* 16: 5077–5086, 2005.
53. Koshiba T, Detmer SA, Kaiser JT, Chen H, McCaffery JM, and Chan DC. Structural basis of mitochondrial tethering by mitofusin complexes. *Science* 305: 858–862, 2004.
54. Lawson VH, Graham BV, and Flanigan KM. Clinical and electrophysiologic features of CMT2A with mutations in the mitofusin 2 gene. *Neurology* 65: 197–204, 2005.
55. Legros F, Lombes A, Frachon P, and Rojo M. Mitochondrial fusion in human cells is efficient, requires the inner membrane potential, and is mediated by mitofusins. *Mol Biol Cell* 13: 4343–4354, 2002.
56. Li X and Gould SJ. The dynamin-like GTPase DLP1 is essential for peroxisome division and is recruited to peroxisomes in part by PEX11. *J Biol Chem* 278: 17012–17020, 2003.
57. Mancini M, Anderson BO, Caldwell E, Sedghinasab M, Paty PB, and Hockenbery DM. Mitochondrial proliferation and paradoxical membrane depolarization during terminal differentiation and apoptosis in a human colon carcinoma cell line. *J Cell Biol* 138: 449–469, 1997.
58. Meeusen S, DeVay R, Block J, Cassidy-Stone A, Wayson S, McCaffery JM, and Nunnari J. Mitochondrial inner-membrane fusion and crista maintenance requires the dynamin-related GTPase Mgm1. *Cell* 127: 2006.
59. Meeusen S, McCaffery JM, and Nunnari J. Mitochondrial fusion intermediates revealed in vitro. *Science* 305: 1747–1752, 2004.
60. Mingrone G, Manco M, Calvani M, Castagneto M, Naon D, and Zorzano A. Could the low level of expression of the gene en-

coding skeletal muscle mitofusin-2 account for the metabolic inflexibility of obesity? *Diabetologia* 48: 2108–2114, 2005.
61. Mozdy AD, McCaffery JM, and Shaw JM. Dnm1p GTPase-mediated mitochondrial fission is a multi-step process requiring the novel integral membrane component Fis1p. *J Cell Biol* 151: 367–380, 2000.
62. Nakamura N, Kimura Y, Tokuda M, Honda S, and Hirose S. MARCH-V is a novel mitofusin 2- and Drp1-binding protein able to change mitochondrial morphology. *EMBO Rep* 7: 1019–1022, 2006.
63. Neuspiel M, Zunino R, Gangaraju S, Rippstein P, and McBride HM. Activated Mfn2 signals mitochondrial fusion, interferes with Bax activation and reduces susceptibility to radical induced depolarization. *J Biol Chem* 280: 25060–25070, 2005.
64. Niemann A, Ruegg M, La PV, Schenone A, and Suter U. 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, 2005.
65. Nowikovsky K, Froschauer EM, Zsurka G, Samaj J, Reipert S, Kolisek M, Wiesenberger G, and Schweyen RJ. The LETM1/YOL027 gene family encodes a factor of the mitochondrial K+ homeostasis with a potential role in the Wolf-Hirschhorn syndrome. *J Biol Chem* 279: 30307–30315, 2004.
66. Olichon A, Baricault L, Gas N, Guillou E, Valette A, Belenguer P, and Lenaers G. Loss of OPA1 perturbates the mitochondrial inner membrane structure and integrity, leading to cytochrome c release and apoptosis. *J Biol Chem* 278: 7743–7746, 2003.
67. Olichon A, Guillou E, Delettre C, Landes T, Rnaune-Pelloquin L, Emorine LJ, Mils V, Daloyau M, Hamel C, Mati-Bonneau P, Bonneau D, Reynier P, Lenaers G, and Belenguer P. Mitochondrial dynamics and disease, OPA1. *Biochim Biophys Acta* 1763: 500–509, 2006.
68. Palade G. Intracellular aspects of the process of protein synthesis. *Science* 189: 347–358, 1975.
69. Pawlikowska P, Gajkowska B, and Orzechowski A. Mitofusin 2 (Mfn2): a key player in insulin-dependent myogenesis in vitro. *Cell Tissue Res* 327: 571–581, 2007.
70. Pedrola L, Espert A, Wu X, Claramunt R, Shy ME, and Palau F. GDAP1, the protein causing Charcot-Marie-Tooth disease type 4A, is expressed in neurons and is associated with mitochondria. *Hum Mol Genet* 14: 1087–1094, 2005.
71. Pich S, Bach D, Briones P, Liesa M, Camps M, Testar X, Palacin M, and Zorzano A. The Charcot-Marie-Tooth type 2A gene product, Mfn2, up-regulates fuel oxidation through expression of OXPHOS system. *Hum Mol Genet* 14: 1405–1415, 2005.
72. Pitts KR, Yoon Y, Krueger EW, and McNiven MA. The dynamin-like protein DLP1 is essential for normal distribution and morphology of the endoplasmic reticulum and mitochondria in mammalian cells. *Mol Biol Cell* 10: 4403–4417, 1999.
73. Praefcke GJ and McMahon HT. The dynamin superfamily: universal membrane tubulation and fission molecules? *Nat Rev Mol Cell Biol* 5: 133–147, 2004.
74. Rizzuto R, Brini M, Murgia M, and Pozzan T. Microdomains with high Ca^{2+} close to IP3-sensitive channels that are sensed by neighboring mitochondria. *Science* 262: 744–747, 1993.
75. Rizzuto R, Pinton P, Carrington W, Fay FS, Fogarty KE, Lifshitz LM, Tuft RA, and Pozzan T. Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca^{2+} responses. *Science* 280: 1763–1766, 1998.
76. Rojo M, Legros F, Chateau D, and Lombes A. Membrane topology and mitochondrial targeting of mitofusins, ubiquitous mammalian homologs of the transmembrane GTPase Fzo. *J Cell Sci* 115: 1663–1674, 2002.
77. Santel A, Frank S, Gaume B, Herrler M, Youle RJ, and Fuller MT. Mitofusin-1 protein is a generally expressed mediator of mitochondrial fusion in mammalian cells. *J Cell Sci* 116: 2763–2774, 2003.
78. Santel A and Fuller MT. Control of mitochondrial morphology by a human mitofusin. *J Cell Sci* 114: 867–874, 2001.
79. Schlickum S, Moghekar A, Simpson JC, Steglich C, O'Brien RJ, Winterpacht A, and Endele SU. LETM1, a gene deleted in Wolf-Hirschhorn syndrome, encodes an evolutionarily conserved mitochondrial protein. *Genomics* 83: 254–261, 2004.
80. Schmidt A, Wolde M, Thiele C, Fest W, Kratzin H, Podtelejnikov AV, Witke W, Huttner WB, and Soling HD. Endophilin I mediates synaptic vesicle formation by transfer of arachidonate to lysophosphatidic acid. *Nature* 401: 133–141, 1999.
81. Schrader M. Shared components of mitochondrial and peroxisomal division. *Biochim Biophys Acta* 1763: 531–541, 2006.
82. Scorrano L, Ashiya M, Buttle K, Weiler S, Oakes SA, Mannella CA, and Korsmeyer SJ. A distinct pathway remodels mitochondrial cristae and mobilizes cytochrome c during apoptosis. *Dev Cell* 2: 55–67, 2002.
83. Shibata Y, Voeltz GK, and Rapoport TA. Rough sheets and smooth tubules. *Cell* 126: 435–439, 2006.
84. Smirnova E, Griparic L, Shurland DL, and van der Bliek AM. Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells. *Mol Biol Cell* 12: 2245–2256, 2001.
85. Smirnova E, Shurland D-L, Ryazantsev SN, and van der Bliek AM. A human dynamin-related protein controls the distribution of mitochondria. *J Cell Biol* 143: 351–358, 1998.
86. Song Z, Chen H, Fiket M, Alexander C, and Chan DC. OPA1 processing controls mitochondrial fusion and is regulated by mRNA splicing, membrane potential, and Yme1L. *J Cell Biol* 178: 749–755, 2007.
87. Stowers RS, Megeath LJ, Gorska-Andrzejak J, Meinertzhagen IA, and Schwarz TL. Axonal transport of mitochondria to synapses depends on milton, a novel *Drosophila* protein. *Neuron* 36: 1063–1077, 2002.
88. Sugioka R, Shimizu S, and Tsujimoto Y. Fzo1, a protein involved in mitochondrial fusion, inhibits apoptosis. *J Biol Chem* 279: 52726–52734, 2004.
89. Suter U and Scherer SS. Disease mechanisms in inherited neuropathies. *Nat Rev Neurosci* 4: 714–726, 2003.
90. Teranishi M, Karbowski M, Kurono C, Nishizawa Y, Usukura J, Soji T, and Wakabayashi T. Effects of coenzyme Q10 on changes in the membrane potential and rate of generation of reactive oxygen species in hydrazine- and chloramphenicol-treated rat liver mitochondria. *Arch Biochem Biophys* 366: 157–167, 1999.
91. Torres M and Forman HJ. Redox signaling and the MAP kinase pathways. *Biofactors* 17: 287–296, 2003.
92. Vanhorebeek I, De Vos R, Mesotten D, Wouters PJ, Wolf-Peeters C, and Van den BG. Protection of hepatocyte mitochondrial ultrastructure and function by strict blood glucose control with insulin in critically ill patients. *Lancet* 365: 53–59, 2005.
93. Vincent AM, Russell JW, Low P, and Feldman EL. Oxidative stress in the pathogenesis of diabetic neuropathy. *Endocr Rev* 25: 612–628, 2004.
94. Voeltz GK, Prinz WA, Shibata Y, Rist JM, and Rapoport TA. A class of membrane proteins shaping the tubular endoplasmic reticulum. *Cell* 124: 573–586, 2006.
95. Voeltz GK, Rolls MM, and Rapoport TA. Structural organization of the endoplasmic reticulum. *EMBO Rep* 3: 944–950, 2002.
96. Yonashiro R, Ishido S, Kyo S, Fukuda T, Goto E, Matsuki Y, Ohmura-Hoshino M, Sada K, Hotta H, Yamamura H, Inatome R, and Yanagi S. A novel mitochondrial ubiquitin ligase plays a critical role in mitochondrial dynamics. *EMBO J*. 25: 3618–3626, 2006.
97. Yoon Y, Krueger EW, Oswald BJ, and McNiven MA. 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, 2003.
98. Yu T, Robotham JL, and Yoon Y. Increased production of reactive oxygen species in hyperglycemic conditions requires dynamic change of mitochondrial morphology. Proc Natl Acad Sci U S A 103: 2653–2658, 2006.
99. Zollino M, Lecce R, Fischetto R, Murdolo M, Faravelli F, Selicorni A, Butte C, Memo L, Capovilla G, and Neri G. Mapping the Wolf-Hirschhorn syndrome phenotype outside the currently accepted WHS critical region and defining a new critical region, WHSCR-2. *Am J Hum Genet* 72: 590–597, 2003.
100. 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, and Vance JM. Axonal neuropathy with

optic atrophy is caused by mutations in mitofusin 2. *Ann Neurol* 59: 276–281, 2006.
101. Zuchner S, Mersiyanova IV, Muglia M, Bissar-Tadmouri N, Rochelle J, Dadali EL, Zappia M, Nelis E, Patitucci A, Senderek J, Parman Y, Evgrafov O, Jonghe PD, Takahashi Y, Tsuji S, Pericak-Vance MA, Quattrone A, Battaloglu E, Polyakov AV, Timmerman V, Schroder JM, and Vance JM. Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. *Nat Genet* 36: 449–451, 2004.
102. Zuchner S and Vance JM. Mechanisms of disease: a molecular genetic update on hereditary axonal neuropathies. *Nat Clin Pract Neurol* 2: 45–53, 2006.

Address reprint requests to:
Luca Scorrano
Dulbecco-Telethon Institute
Venetian Institute of Molecular Medicine
Via Orus 2
35129 Padova, Italy

E-mail: lscorrano@dti.telethon.it

Date of first submission to ARS Central, September 27, 2007; date of final revised submission, October 3, 2007; date of acceptance, October 3, 2007.

This article has been cited by:

1. Sang-Bing Ong , Andrew R. Hall , Derek J. Hausenloy . Mitochondrial Dynamics in Cardiovascular Health and Disease. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
2. Melissa K. McCoy , Mark R. Cookson . 2012. Mitochondrial Quality Control and Dynamics in Parkinson's Disease. *Antioxidants & Redox Signaling* **16**:9, 869-882. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
3. Christelle Kamga, Suhas Krishnamurthy, Sruti Shiva. 2012. Myoglobin and mitochondria: A relationship bound by oxygen and nitric oxide. *Nitric Oxide* **26**:4, 251-258. [CrossRef]
4. Cecilia Bucci, Oddmund Bakke, Cinzia Progida. 2012. Charcot–Marie–Tooth disease and intracellular traffic. *Progress in Neurobiology* . [CrossRef]
5. Björn Oettinghaus, Maria Licci, Luca Scorrano, Stephan Frank. 2011. Less than perfect divorces: dysregulated mitochondrial fission and neurodegeneration. *Acta Neuropathologica* . [CrossRef]
6. L. Ramió-Lluch, J.M. Fernández-Novell, A. Peña, D. Bucci, T. Rigau, J.E. Rodríguez-Gil. 2011. “In vitro” capacitation and subsequent acrosome reaction are related to changes in the expression and location of midpiece actin and mitofusin-2 in boar spermatozoa. *Theriogenology* . [CrossRef]
7. Pascal Pierre, Ivan R. Nabi. 2011. The Gp78 ubiquitin ligase: probing endoplasmic reticulum complexity. *Protoplasma* . [CrossRef]
8. Zuoguang Wang, Ya Liu, Jieling Liu, Kuo Liu, Jie Wen, Shaojun Wen, Zhaosu Wu. 2011. HSG/Mfn2 Gene Polymorphism and Essential Hypertension: a Case-Control Association Study in Chinese. *Journal of Atherosclerosis and Thrombosis* **18**:1, 24-31. [CrossRef]
9. S.-B. Ong, D. J. Hausenloy. 2010. Mitochondrial morphology and cardiovascular disease. *Cardiovascular Research* **88**:1, 16-29. [CrossRef]
10. Silvia Campello, Luca Scorrano. 2010. Mitochondrial shape changes: orchestrating cell pathophysiology. *EMBO reports* **11**:9, 678-684. [CrossRef]
11. Amadou K.S. Camara , Edward J. Lesnefsky , David F. Stowe . 2010. Potential Therapeutic Benefits of Strategies Directed to Mitochondria. *Antioxidants & Redox Signaling* **13**:3, 279-347. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
12. Fred Y. Xu, Heidi McBride, Devrim Acehan, Frédéric M. Vaz, Riekelt H. Houtkooper, Raymond M. Lee, Michael A. Mowat, Grant M. Hatch. 2010. The dynamics of cardiolipin synthesis post-mitochondrial fusion. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1798**:8, 1577-1585. [CrossRef]
13. E. Flores, J.M. Fernández-Novell, A. Peña, T. Rigau, J.E. Rodríguez-Gil. 2010. Cryopreservation-induced alterations in boar spermatozoa mitochondrial function are related to changes in the expression and location of midpiece mitofusin-2 and actin network. *Theriogenology* **74**:3, 354-363. [CrossRef]
14. Werner J.H. Koopman , Leo G.J. Nijtmans , Cindy E.J. Dieteren , Peggy Roestenberg , Federica Valsecchi , Jan A.M. Smeitink , Peter H.G.M. Willems . 2010. Mammalian Mitochondrial Complex I: Biogenesis, Regulation, and Reactive Oxygen Species Generation. *Antioxidants & Redox Signaling* **12**:12, 1431-1470. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
15. L Ciarlo, V Manganelli, T Garofalo, P Matarrese, A Tinari, R Misasi, W Malorni, M Sorice. 2010. Association of fission proteins with mitochondrial raft-like domains. *Cell Death and Differentiation* **17**:6, 1047-1058. [CrossRef]
16. Eva Trevisson, Leonardo Salviati, Luca ScorranoOPA1 and Its Clinical Implications . [CrossRef]
17. Axel Niemann, Konstanze Marion Wagner, Marcel Ruegg, Ueli Suter. 2009. GDAP1 mutations differ in their effects on mitochondrial dynamics and apoptosis depending on the mode of inheritance. *Neurobiology of Disease* **36**:3, 509-520. [CrossRef]
18. Matteo Landriscina , Francesca Maddalena , Gabriella Laudiero , Franca Esposito . 2009. Adaptation to Oxidative Stress, Chemoresistance, and Cell Survival. *Antioxidants & Redox Signaling* **11**:11, 2701-2716. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
19. Tina Wenz. 2009. PGC-1Î± activation as a therapeutic approach in mitochondrial disease. *IUBMB Life* **61**:11, 1051-1062. [CrossRef]
20. Peter H.G.M. Willems, Jan A.M. Smeitink, Werner J.H. Koopman. 2009. Mitochondrial dynamics in human NADH:ubiquinone oxidoreductase deficiency. *The International Journal of Biochemistry & Cell Biology* **41**:10, 1773-1782. [CrossRef]

21. Luca Scorrano. 2009. Opening the doors to cytochrome c: Changes in mitochondrial shape and apoptosis. *The International Journal of Biochemistry & Cell Biology* **41**:10, 1875-1883. [CrossRef]
22. Petr Ježek, Lydie Plecitá-Hlavatá. 2009. Mitochondrial reticulum network dynamics in relation to oxidative stress, redox regulation, and hypoxia. *The International Journal of Biochemistry & Cell Biology* **41**:10, 1790-1804. [CrossRef]
23. John J. LemastersMolecular Mechanisms of Cell Death 3-24. [CrossRef]
24. Annalisa Carlucci, Luca Lignitto, Antonio Feliciello. 2008. Control of mitochondria dynamics and oxidative metabolism by cAMP, AKAPs and the proteasome. *Trends in Cell Biology* **18**:12, 604-613. [CrossRef]