Physiol Rev 89: 799–845, 2009;
doi:10.1152/physrev.00030.2008.

Mitochondrial Dynamics in Mammalian Health and Disease

MARC LIESA, MANUEL PALACÍN, AND ANTONIO ZORZANO

Institute for Research in Biomedicine (IRB Barcelona), CIBER de Diabetes y Enfermedades Metabólicas Asociadas, and Departament de Bioquímica i Biologia Molecular, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain

Liesa M, Palacín M, Zorzano A. Mitochondrial Dynamics in Mammalian Health and Disease. *Physiol Rev* 89: 799–845, 2009; doi:10.1152/physrev.00030.2008.—The meaning of the word *mitochondrion* (from the Greek *mitos*, meaning thread, and *chondros*, grain) illustrates that the heterogeneity of mitochondrial morphology has been known since the first descriptions of this organelle. Such a heterogeneous morphology is explained by the dynamic nature of mitochondria. Mitochondrial dynamics is a concept that includes the movement of mitochondria along the cytoskeleton, the regulation of mitochondrial architecture (morphology and distribution), and connectivity mediated by tethering and fusion/fission events. The relevance of these events in mitochondrial and cell physiology has been partially unraveled after the identification of the genes responsible for mitochondrial fusion and fission. Furthermore, during the last decade, it has been identified that mutations in two mitochondrial fusion genes (*MFN2* and *OPA1*) cause prevalent neurodegenerative diseases (Charcot-Marie Tooth type 2A and Kjer disease/autosomal dominant optic atrophy). In addition, other diseases such as type 2 diabetes or vascular proliferative disorders show impaired MFN2 expression. Altogether, these findings have established mitochondrial dynamics as a consolidated area in cellular physiology. Here we review the most significant findings in the field of mitochondrial dynamics in mammalian cells and their implication in human pathologies.

I. INTRODUCTION: MITOCHONDRIAL DYNAMICS

The heterogeneity of mitochondrial morphology has been known since the early descriptions of this organelle in the mid 1800s. Mitochondria were described for the first time in 1857, by Rudolph Albert Von Kölliker, as granular cytoplasmic compartments with their own membrane present in muscle (referred to as *sarcosomes* by Kölliker). Later on, several studies giving distinct names

to the same organelle were published. Among these, in 1898 Benda coined it with the name *mitochondrion* (26), coming from the Greek words *mitos* (meaning thread) and *chondron* (grain). This term considers the most common morphological features of this organelle and was broadly used and accepted to describe this granule from the end of the 1930s onwards (184).

At the beginning of the 20th century, advances in bright-field microscopy and the availability of well-spread cultured cells from chicken allowed the first reliable observations of mitochondrial shape changes and dynamics in living cells, possibly caused by fusion and fission events of these organelles (186). In addition, in 1931, this dynamism was also demonstrated to be developmentally regulated in liver (275). However, less attention was paid to this mitochondrial feature, as during the 1940s and 1950s the autonomous biochemical function of mitochondria was discovered, together with the demonstration that β-oxidation, the Krebs cycle, and oxidative phosphorylation (OXPHOS) occurred inside these organelles (184). Furthermore, in the 1950s, Palade and Sjöstrand published high-resolution electron microscopy images of mitochondria, which elucidated the ultrastructural features of this granule and showed it as an isolated organelle (Fig. 1*A*). Therefore, these images indicated an apparent lack of physical and functional connection between distinct mitochondria. This concept of functional independency was favored by the discovery, in the 1960s, of mitochondrial DNA and, afterwards, its bacterial origin, thereby confirming the bacterial endosymbiotic theory of mitochondrial origin, postulated by Altmann in 1890 (5). Several reports in the late 1960s, 1970s, and early 1980s went against this independency, as mitochondrial DNA complementation and recombination were demonstrated in yeast (294). These mitochondrial DNA events were postulated to result from fusion events between mitochondria in this organism (212, 282, 283). In the same period, there were further descriptions of mitochondrial fusion, mitochondrial networks, and mega-mitochondria formation in differentiated mammalian tissues, such as liver and muscle (14, 36, 168, 311, 312), and also of a developmentally regulated mitochondrial network in rat skeletal muscle (18, 19).

In the 1990s, time-lapse analysis using mitochondrial-targeted fluorescent proteins and dyes allowed detailed and dynamic visualization of mitochondrial shape changes and structure rearrangements compatible with fusion events in living cells (from yeast to hepatocytes) (Fig. 1*B*) (27, 65, 66, 221, 251). Moreover, electron tomography analysis unraveled additional mitochondrial inner membrane structures, such as cristae junctions, and their dynamic regulation (197, 198). In addition, the first gene that participates in the fusion of mitochondria was identified in *Drosophila* (123). These findings, among others, led to the discovery of a group of genes that control and execute mitochondrial fusion and fission, most of them first identified in yeast (42, 204, 276, 322).

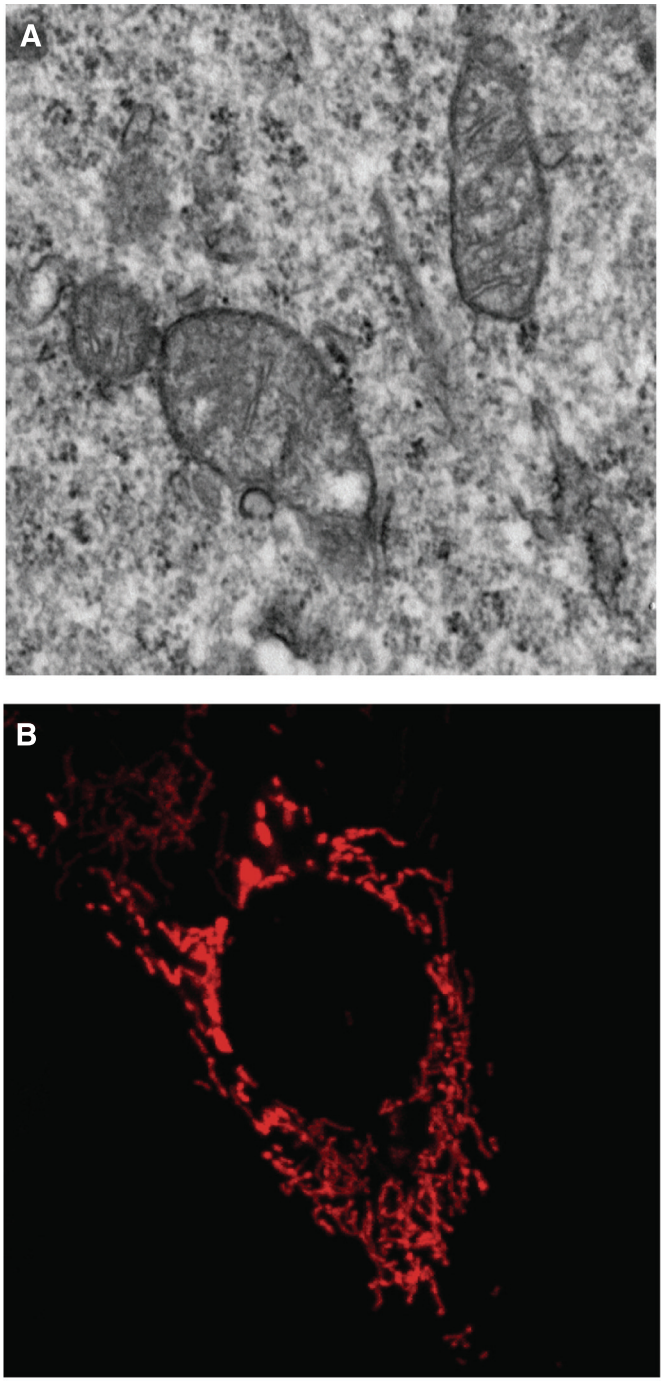


FIG. 1. Visualization of mitochondria by electron or fluorescence microscopy. *A*: image of three mitochondria obtained at ×40,000 magnification from murine myoblasts by electron microscopy. *B*: single frame of a time-lapse analysis of myoblasts stably expressing a mitochondrial-targeted red fluorescent protein and visualized by confocal microscopy (objective ×63). The dynamics of the whole mitochondrial network is observed in myoblasts with this latter technique, in contrast to electron microscopy images.

The use of mitochondrial-targeted fluorescent proteins and dyes also allowed the static visualization of mitochondrial architecture (defined by morphology and distribution within the cell, i.e., mitochondrial network). The modulation of mitochondrial architecture has been broadly interpreted as evidence of altered mitochondrial fusion and/or fission activity. However, precise conclusions regarding the rates of these two processes cannot be made on the basis of mitochondrial architecture alone. For instance, equivalent mitochondrial architecture can be detected in response to increased mitochondrial connectivity (caused by tethering or fusion) or to the inhibition of mitochondrial fission (Fig. 2), as the use of these proteins and dyes does not allow the visualization of the exchange of mitochondrial matrix proteins that results mitochondrial fusion.

Therefore, to measure rates of mitochondrial protein exchange, PEG-mediated cell fusion assays have been used (182). The basis for this assay is the induction of fusion between two separate cell populations whose mitochondrial matrix is labeled in a different manner (i.e., with green and red mitochondrial-targeted fluorescent proteins). The distinct mitochondrial labels in fused cells (polykarions) colocalize only when there are mitochondrial fusion events that cause the exchange of mitochondrial matrix proteins. However, this methodology to measure mitochondrial fusion rates cannot be applied successfully to all cellular models and does not measure the degree of mitochondrial connectivity. Thus the next step forward has been the use of photoactivable mitochondrial matrix-targeted fluorescent proteins. These proteins improved the precision of the measurement of mitochondrial fusion rates and connectivity. The photoactivation of these proteins, within a small region of the mitochondrial matrix, allows fused mitochondria to be differentiated from nonfused ones (or only tethered), as the activated protein diffuses and spreads the fluorescent signal only through a continuous matrix of fused mitochondria (303). Moreover, the rate of dilution of the fluorescent signal (due to fusion events with unlabeled mitochondria) can also be used as a measure of mitochondrial fusion rates (157). Importantly, an additional method that allows the measurement of mitochondrial fusion in isolated mitochondria (in vitro) has also been successfully applied (205).

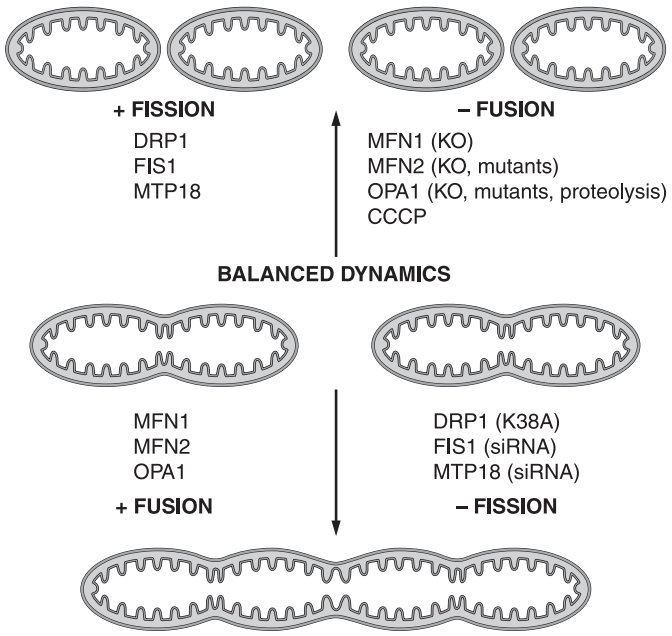


FIG. 2. Mitochondrial morphology is partly dependent on a proper balance between fusion and fission processes. Schematic representation of mitochondrial morphology is shown in response to modulation of the activity of proteins involved in mitochondrial fusion or fission. Mammalian mitochondrial fusion is mediated mainly by Mfn1, Mfn2, and Opa1. Reduction in the activity of these proteins by repression or by the presence of loss-of-function mutants or ablation of these proteins causes mitochondrial fragmentation or reduction in the extent of mitochondrial filaments (12, 16, 51, 92, 115, 223). In contrast, reexpression of Mfn1, Mfn2, or OPA1 in cells with prior deficient activity of these any of these proteins promotes the formation of mitochondrial filaments (59, 72, 277). Furthermore, gain-of-function of some of these proteins in the presence of abolished mitochondrial fission results in long mitochondrial filaments (256). Mammalian mitochondrial fission is mediated by Drp1, Fis1, and MTP18. Reduction in the activity of these proteins by repression or by mutation, or ablation of these proteins causes elongation of mitochondrial network (97, 148, 240, 274, 285, 299, 304, 325, 327). In contrast, overexpression of these proteins causes mitochondrial fragmentation (148, 285, 298, 325).

In addition, several relevant molecular mechanisms by which genes controlling mitochondrial dynamics promote mitochondrial fusion or fission and the posttranscriptional regulation of these genes have been elucidated. Furthermore, the first functional evidence demonstrating that the genes involved in mitochondrial dynamics contribute to oxidative metabolism, apoptosis, autophagy, and cell cycle has been gathered during the last decade.

However, the overall process of mitochondrial fusion and fission has not been thoroughly defined, and it is likely that a number of genes executing or modulating these activities remain to be determined. In fact, all these studies have been done mostly in yeast and cultured mammalian cells (mainly undifferentiated or transformed cells). Thus the pattern, physiological relevance, and regulation of mitochondrial dynamics in human or mouse tissues, such as skeletal muscle or liver, are poorly understood or unknown. Indeed, there is some evidence demonstrating that mitochondrial dynamics is specifically regulated depending on the cell type or on the given function of certain tissues. For instance, mitochondrial architecture in mouse pancreatic acinar (exocrine) cells is specifically suited to permit delimitated increases in cytosolic calcium concentrations only in the apical region of the cell, which is required for normal exocytosis of apical granules, i.e., for their specific exocrine function (296). Another good example of this specific regulation is the concerted distribution of mitochondria and sarcoplasmic reticulum, which contributes to the type of contrac-

tion, relaxation, and calcium fluxes in distinct muscle fibers (99). Importantly, the factors that control and determine these differences in mitochondrial dynamics or architecture among distinct cells lines (63), cell types, or tissues have not been determined. The identification of these factors will be crucial not only to explain the differences in mitochondrial architecture and dynamics between tissues, but may also provide the basis for understanding tissue-specific differences of mitochondrial function, its regulation, and pathogenic implications.

To date, a biochemical method that allows the quantification of mitochondrial fusion or fission rates has not been described. Such a method would facilitate the testing or identification, through high-throughput screenings, of hypothetical drugs that modulate these rates. Furthermore, the discovery of a mammalian biomarker of mitochondrial fusion and fission would allow researchers to determine the defects on these processes in patients or in diseases where a defect in the same is not described. Unraveled only in yeast, this biomarker has been used to find a chemical compound that inhibits mitochondrial division. This biomarker is based on the effect of changes in mitochondrial fusion and fission rates on *Saccharomyces cerevisae* proliferation (45). A similar biomarker in mammals, together with the finding of a biochemical method to measure mitochondrial fission and fusion rates, would be a significant step forward to find a cure for mitochondrial dynamics-related diseases.

Here we review current knowledge of mitochondrial dynamics in mammals, focusing mainly on its role in cell and mitochondrial function and its involvement in pathological processes. Regarding the specific details of mitochondrial dynamics machinery in yeast, readers are referred to these excellent reviews (133, 222, 246, 249, 266, 318, 322).

II. REGULATION OF MITOCHONDRIAL FUSION: PROTEINS INVOLVED IN MITOCHONDRIAL FUSION IN MAMMALIAN CELLS

It has been widely demonstrated that changes in the activity of fusion and fission proteins result in alterations in mitochondrial shape (Fig. 2). Indeed, when both fusion and fission are inhibited by the modulation of these proteins, mitochondrial morphology is similar to that observed in a basal state, thereby demonstrating that shape is a consequence of the balance between these two processes (51) (Fig. 2). Under conditions of enhanced fission, mitochondria fuse at a normal or basal rate (196). Nevertheless, although the shape of these organelles is indicative of the balance between mitochondrial fusion and fission in most situations, it is not a definitive parameter to conclude enhanced fusion or fission rates. Thus mitochondrial morphology depends not only on components of mitochondrial dynamics (that is to say proteins that mediate mitochondrial fusion or fission), but may also greatly rely on distinct proteins or mediators, such as cytoskeleton proteins, the "railway" used by mitochondria to move inside the cell (11), or other unknown cellular components. In this regard, changes in the activity of mitochondrial fusion proteins also modulate the distribution and movement of this organelle (50, 51, 236, 252, 255, 256), in addition to shape. These observations therefore suggest cross-talk between these unknown and/or cytoskeletal components and mitochondrial fusion proteins.

Mitochondrial fusion is a two-step process, where the outer and inner mitochondria fuse by separable events (196). This process can be explained by the finding that the most relevant proteins described to date that are involved in mammalian mitochondrial fusion show distinct mitochondrial sublocalization: mitofusin 1 and 2 (MFN1 and MFN2, respectively, located on the outer mitochondrial membrane and can explain outer membrane fusion) and OPA1 (located in the inner membrane and intermembrane space, which can explain inner membrane fusion). However, a high-resolution mechanism of how the outer and inner mitochondrial membranes coordinately fuse and the elucidation of the factors that signal to a particular region of the mitochondrial membrane to start the tethering and fusion and then to correctly distribute mitochondrial content remain to be determined.

To gain insight into these processes, the study of the proteins controlling mitochondrial fusion and fission, their activity, and regulation is crucial. In this section, we discuss the most relevant mammalian proteins described to date to be involved in mitochondrial fusion, the aforementioned MFN1, MFN2, and OPA1.

A. MFN1

MFN1 was identified in mammals, together with MFN2, as the human homologs of *Drosophila* Fuzzy onions protein (Fzo, key for spermatogenesis), the first gene discovered to mediate mitochondrial fusion (123, 256). Human MFN1 is significantly conserved throughout chordates (Fig. 3). Human MFN1 protein shows 63% of identity with MFN2, and both proteins share the same relevant functional domains (Table 1) (256). This MFN1/MFN2 identity is maintained, in different percentages, through chordates, from human to zebrafish (Table 1). Importantly, in NCBI databases, *Ciona intestinalis* (an ancient chordate species) shows only one gene similar to MFN. This finding suggests that MFN1 and MFN2 have a common ancestor similar to that found in *Ciona intestinalis* (Tables 1 and 2 and Fig. 3). Analysis of other species reveals the existence of only one gene in fungi (*Saccharomyces* and *Podospora*), one MFN ortholog gene in *Caenorhabditis elegans*, and two genes in *Drosophila melanogaster* (Fzo is specific to the male germ line and is not

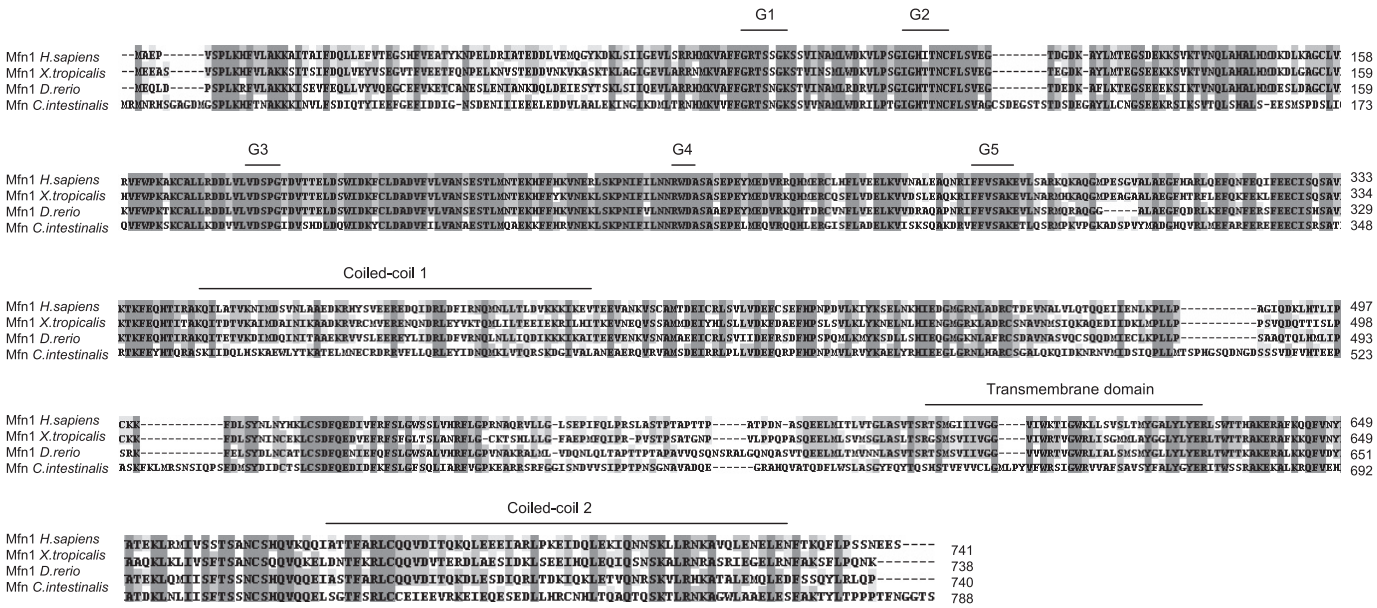


FIG. 3. Alignment of Mfn1 sequences from several chordate species and the ancient chordate *Ciona intestinalis*. Alignments were performed with Clustal W on-line software. Residues are shown in intensities of gray in function of their identity through *Homo sapiens*, *Danio rerio* (zebrafish), *Xenopus tropicalis* (frog), and *Ciona intestinalis* (ancient chordate). Dark gray shows the residues found in all the species analyzed and light gray the residues found only in some of them. The human MFN1 protein domains are shown. Sequences used are as follows: MFN1 *H. sapiens* (NP_284941), Mfn1 *D. rerio* (NP_956941), Mfn1 *X. tropicalis* (CAJ83518), and Mfn *C. intestinalis* (XP_002126852).

expressed in female embryos or adults, whereas Mfn is expressed in adult male or female tissues and its identity to MFN is higher compared with Fzo) (141) and two genes in *Trichoplax adhaerens* (Tables 1 and 2).

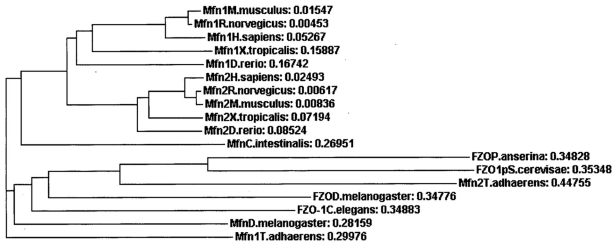
1. Protein domains and activity

MFN1 is a transmembrane GTPase protein of 741 residues in humans and is located in the outer mitochondrial membrane. This localization requires the COOH-terminal part of the protein [residues 419-741 amino acids (aa)], which contains a transmembrane domain (residues 596-630 aa) and a coiled-coil domain 2 (also called heptad-repeat domain; HR2, residues 673-728 aa) (Figs. 4 and 5*A*) (255). This heptad-repeat domain mediates the first step of mitochondrial fusion, namely, the tethering of two adjacent mitochondria through a dimeric antiparallel coiled-coil structure (175) (Fig. 5*B*). These dimers or complexes can be homotypic (Mfn1-Mfn1) or heterotypic (Mfn1-Mfn2) (51, 175). The distinct proportion of homo- and heterotypic complexes may be relevant for the rate of mitochondrial fusion activity in the cell, as shown by the higher GTPase activity, with lower affinity to GTP, exhibited by purified Mfn1 compared with Mfn2 (144). In this regard, Mfn1-harboring mitochondria show higher tethering efficiency than those harboring Mfn2 (144). The formation of these three complexes leads to the notion that the rates of Mfn-mediated mitochondrial fusion differ in distinct tissues, as the MFN2 expression pattern is more tissue specific than MFN1, although both are widely expressed (16, 51, 252, 255). Of note, skeletal muscle has the highest MFN2/MFN1 ratio (255).

The NH_2-terminal part of the MFN1 protein contains the GTP-binding domain and another coiled-coil domain (HR1, residues 346-401 aa) (175, 255) (Fig. 4). This GTP-binding domain has the five GTPase motifs or boxes previously defined for Ras (34, 35). These motifs are named G1 (82-89 aa), G2 (106-111 aa), G3 (173-177 aa), G4 (236-241 aa), and G5 (281-287 aa) (Figs. 3 and 4). G1 binds the phosphates of the GTP molecule; G3 coordinates the Mg^{2+} required for hydrolysis and G1, G2, and G3 together form the catalytic center; whereas G4 and G5 provide the specific conformation to allow only GTP binding (and not ATP) (34, 35). The GTPase activity of this domain is crucial for MFN1 fusion activity (51, 144, 255), as shown by studies reporting that MFN1 mutants (such as the highly conserved residue K88T in the G1 motif) of this domain lose the capacity to elongate or fuse mitochondria (51, 255). Furthermore, other MFN1 mutants of the same domain (T109A residue in the G2 motif) act as dominant negative forms; that is to say they disturb wild-type or endogenous MFN1 fusion activity (255). In addition, high overexpression of human MFN1 causes a perinuclear aggregation of mitochondria (255). This aggregation seems to be independent of MFN1 activity in mitochondrial fusion, as overexpression of MFN1 GTPase loss-of-function mutants causes mitochondrial aggregation (255). Indeed, expression of a truncated Mfn1 form (deletion from aa 1 to 331, therefore lacking the GTP-binding domain) also induces perinuclear aggregation of mitochondria. Importantly, mutations in the coiled-coil 2 domain that disrupt the Mfn1 dimeric antiparallel coiled-coil structure (such

TABLE 1. *Phylogram tree and Mfn1/Mfn2 identity in chordates and prochordates*

Organism	Mfn1/Mfn2 Percent Identity
H. sapiens	63
R. norvegicus	65
M. musculus	66
X. tropicalis	62
D. rerio	64
*C. intestinalis**	*



Percentages of identity and phylogram were obtained using ClustalW2 on-line software (http://www.ebi.ac.uk/Tools/clustalw2/index.html). MFN1 *H. sapiens* (NP_284941), Mfn1 *M. musculus* (NP_077162), Mfn1 *R. norvegicus* (NP_620432), Mfn1 *D. rerio* (NP_956941), Mfn1 *X. tropicalis* (CAJ83518), Mfn *C. intestinalis* (XP_002126852). MFN2 *H. sapiens* (NP055689), Mfn2 *R. norvegicus* (NP_570964), Mfn2 *M. musculus* (NP_573464), Mfn2 *D. rerio* (AP19405), Mfn2 *X. tropicalis* (CAJ82624), FZO1 *P. anserina* (ABY76319), "similar to MFN1" *T. adhaerens* (EDV23825), "similar to MFN2" *T. adhaerens* (XP_002111215), FZO *D. melanogaster* (NP_73284), Mfn *D. melanogaster* (NP_572320), FZO-1 *C. elegans* (NP_495161.1), Fzo1p *S. cerevisae* (NP_009738.1). *Only one gene similar to Mfn found in *C. intestinalis*.

as mutation of mouse residues L691P or L705P) impede the aggregation induced by this truncated Mfn1 (175). Taken together, these data suggest that mitochondrial tethering, together with the incapacity to fuse mitochondria, is responsible for this mitochondrial aggregation. Interestingly, in addition to the defect in mitochondrial fusion, Mfn1-ablated cells show defective mitochondrial mobility, without apparent alterations in the cytoskeleton (51). Therefore, one may propose that deregulated mitochondrial mobility caused by excessive MFN1 gain-of-function also contributes to perinuclear mitochondrial clustering, in addition to mitochondrial tethering.

Some evidence demonstrates that MFN1 has a posttranscriptional and posttranslational regulation. This has been shown by the identification of alternative splicing isoforms, which were found in lung cancer (56), and the observation that the varying relative protein levels between tissues do not always match the relative mRNA levels (255). However, the molecular mechanisms for this regulation are largely unknown.

2. Physiological role of Mfn1 activity

The physiological relevance of mitochondrial fusion mediated by Mfn1 has been demonstrated in genetically Mfn1-ablated mice. Mfn1 expression is essential for the development of embryos, as Mfn1 loss-of-function causes a lethal placental defect, as does Mfn2 loss-of-function (51, 52). Fibroblasts from Mfn1-ablated mouse embryos show a dramatic reduction in mitochondrial fusion activity, which is more severe than in the case of Mfn2 ablation (51). This difference is in agreement with the higher tethering and GTPase activity observed in Mfn1-harboring mitochondria and purified Mfn1, respectively, compared with Mfn2 (144). Furthermore, the effects of the mouse isoform 8, which is similar to human transcript 1 of optic atrophy gene 1 (Opa1, a dynamin-related protein with GTPase activity, which is discussed later) that promotes mitochondrial fusion, require Mfn1 but not Mfn2 expression in mouse embryonic fibroblasts (59). This observation therefore again suggests a more prominent role of Mfn1 than Mfn2 in mitochondrial fusion.

Despite this prominence in undifferentiated and/or transformed cell lines, conditional Mfn1-ablated mice (Meox2-Cre/Mfn1^{loxP}, in which Mfn1 expression is present only in placental cells, therefore preventing the previously described embryonic lethality in fully Mfn1-ablated mice) are born fully viable and are fertile for at least one year (52). In marked contrast, conditional Mfn2-ablated mice (Meox2-Cre/Mfn2^{loxP}) show movement defects and impaired cerebellar development, with major alteration in Purkinje cells, and 33% die on postnatal day 1 and the rest on postnatal day 17 (52). These specific defects in cerebellar development caused by the lack of Mfn2 and not Mfn1 can be explained by the fact that Purkinje cells show lower expression of Mfn1 mRNA than Mfn2 mRNA, which implies that ablation of cerebellar Mfn2 expression may cause a greater defect in mitochondrial fusion than cerebellar Mfn1 ablation (52). Another explanation for this cerebellar difference could be that Mfn2 has specific mitochondrial or nonmitochondrial activities that are essen-

TABLE 2. *Conservation of mitochondrial fusion proteins (chordates and prochordates)*

Organism and Percent Identity to *H. sapiens*	Mfn1	Mfn2	OPA1
R. norvegicus	90	95	96
M. musculus	89	94	95
X. tropicalis	69	85	88
D. rerio	68	82	79
*C. intestinalis**	46*	49*	55

Percentages of identity were obtained using ClustalW2 on-line software. Sequences used: MFN1 *H. sapiens* (NP_284941), Mfn1 *M. musculus* (NP_077162), Mfn1 *R. norvegicus* (NP_620432), Mfn1 *D. rerio* (NP_956941), Mfn1 *X. tropicalis* (CAJ83518), Mfn *C. intestinalis* (XP_002126852). MFN2 *H. sapiens* (NP055689), Mfn2 *R. norvegicus* (NP_570964), Mfn2 *M. musculus* (NP_573464), Mfn2 *D. rerio* (AP19405), Mfn2 *X. tropicalis* (CAJ82624). OPA1 isoform 1 *H. sapiens* (NP_056375.1), Opa1 *M. musculus* (NP_598513.1), Opa1 *R. norvegicus* (NP_598269.3), Opa1 *D. rerio* (NP_001007299.1), Opa1 *X. tropicalis* (NP_001120510), Opa1 *C. intestinalis* (XP_002131771.1). *Only one gene similar to Mfn found in *C. intestinalis*.

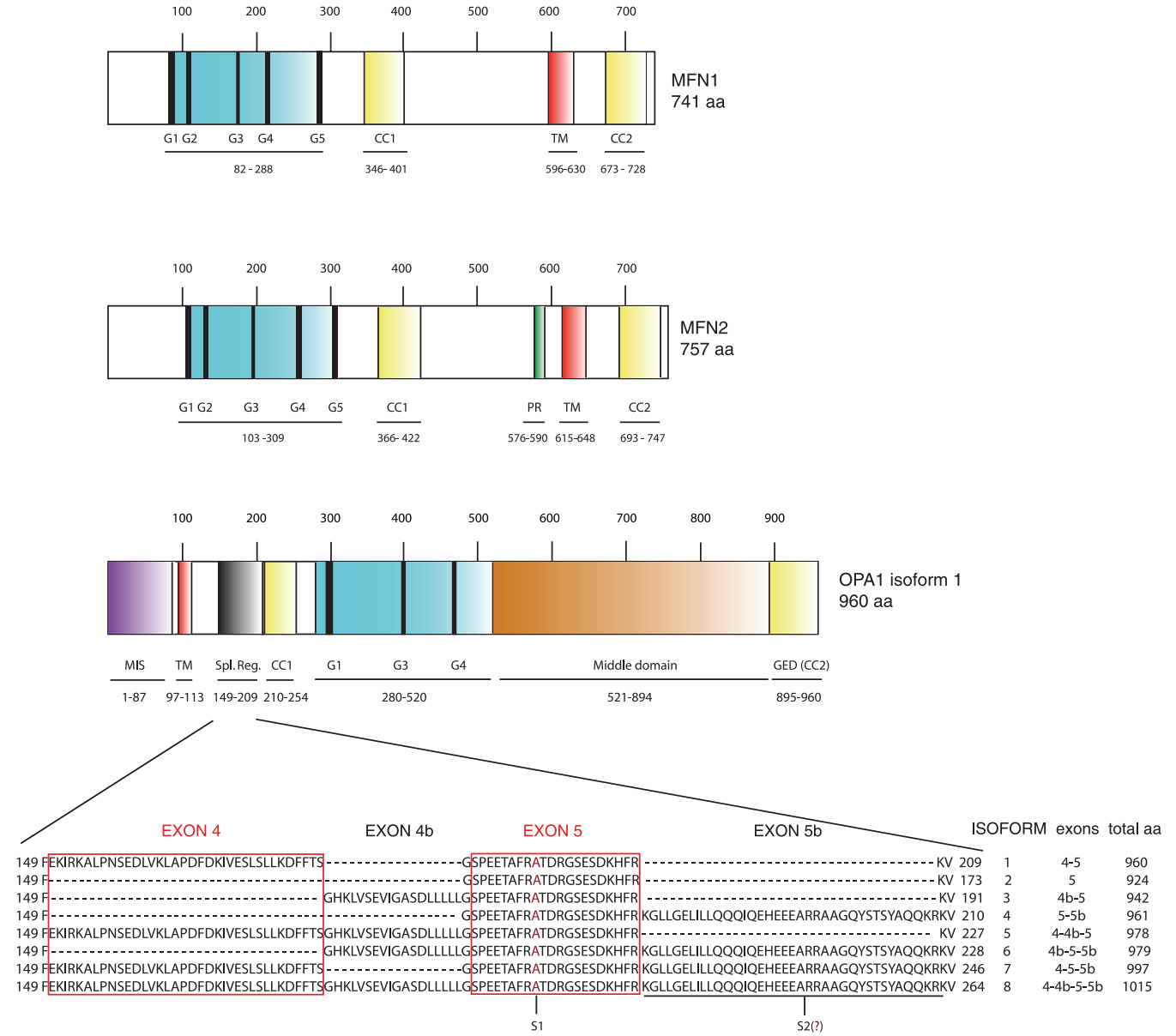


FIG. 4. Domains and motifs identified in human mitochondrial fusion proteins MFN1, MFN2, and OPA1. The GTPase domains are shown in blue with the distinct G motifs shown in black bars (from G1 to G5 depending on the protein). Coiled-coil regions (CC) and/or GED (GTPase effector domain) are shown in yellow. The transmembrane domains (TM) are shown in red. These domains are found in MFN1, MFN2, and OPA1. The proline-rich region (PR, green, involved in protein-protein interactions) is found only in MFN2, poorly conserved in MFN1 and not found in OPA1. The mitochondrial import sequence (MIS) is shown in purple, with the cleavage site of mitochondrial processing protease (MPP) in residue F88. The middle domain is colored in brown and the alternative spliced region (Spl. Reg.) in black. These three regions have been described only in OPA1. The length and topology in amino acids for each region and domain are shown. The primary sequence for each OPA1 splicing isoform is also depicted. The alternative splicing of exons 4, 4b, and 5b generates eight isoforms of OPA1 with distinct numbers of total amino acids. All OPA1 isoforms contain exon 5 with the S1 cleavage site (alanine). Exon 5b contains a second cleavage site S2, although the exact residue of this site has not been determined.

tial for Purkinje cells. Whatever the case, the lack of physiological defects of Mfn1 loss-of-function provides further evidence of the specific relevance of Mfn2 presence in homo- and heterotypic complexes in differentiated adult tissues and of the tissue expression specificity of this protein in mitochondrial fusion and physiology.

However, the absence of significant defects or phenotype in conditional Mfn1-ablated mice (Meox2-Cre/Mfn1^{loxP}) under basal conditions suggests that Mfn1 and/or its mitochondrial fusion activity is required only for the development of the placenta. In addition, this implies that in embryonic/fetal cells or tissues and in the postnatal life, Mfn1 function can be substituted under physiological conditions (probably by Mfn2 or other unknown proteins). Therefore, the biological relevance of Mfn1 in adult mice remains to be determined. In this

regard, it would be of great interest to study: *1*) whether mitochondrial fusion rates are normal in adult tissues from *Mfn1* conditional knockout (KO) mice and, if so, whether this is due to selective compensatory effects that do not occur in cultured cells [which would explain the absence of a phenotype in conditional KO mice under normal conditions but the existence of phenotype in mouse embryonic fibroblasts (MEF)]; *2*) the stoichiometry of Mfn2 versus Mfn1 molecules in distinct adult tissues to establish the extent of a reduction in mitochondrial fusion caused by Mfn2 or Mfn1 loss-of-function; and *3*) the conditions or stimuli that specifically increase Mfn1-mediated mitochondrial fusion, and which may permit the detection of a distinct phenotype in *Mfn1* conditional KO mice.

Several conditions or stimuli have been described to increase Mfn1 expression and could provide clues as to the physiological requirement for Mfn1 activity in adult tissues. These conditions include exercise in muscle and caloric restriction in brain, heart, liver, and white and brown adipose tissue (which also increase mitochondrial biogenesis) (44, 219). Interestingly, during the differentiation of primary neuron cultures, there is a marked decrease in Mfn1 expression. This observation suggests a minor requirement of Mfn1 activity in differentiated neurons (49) and is consistent with the lack of neurodegeneration in conditional Mfn1-ablated mice (52). An increase in extracellular ammonium concentration in CHO cells is another condition that enhances Mfn1 expression, although the physiological relevance of this adaptation remains to be determined (54).

3. Partners and modulators

Mfn1 physically interacts with Mfn2 (51, 78, 175) and with OPA1 (119). As demonstrated by *Mfn1* or *Mfn2* KO mouse phenotypes, both proteins play partially nonredundant and quantitatively distinct roles in mitochondrial fusion in cell lines, in spite of showing ~80% similarity in their primary sequence. To discern the mechanism of nonredundancy or to find new (different or not) roles of these two homologs in cell and mitochondrial physiology, the identification of specific interacting partners or modulators of their activity is crucial.

Mitofusin-binding protein (Mib) is a novel protein that was identified by affinity purification of rat liver cytosol using a column that contained the cytosolic part of rat Mfn1 (91). Therefore, there is an interaction between Mib and the cytosolic part of Mfn1. Mib is a member of the medium-chain reductase protein superfamily, which has a conserved coenzyme-binding domain. Mib

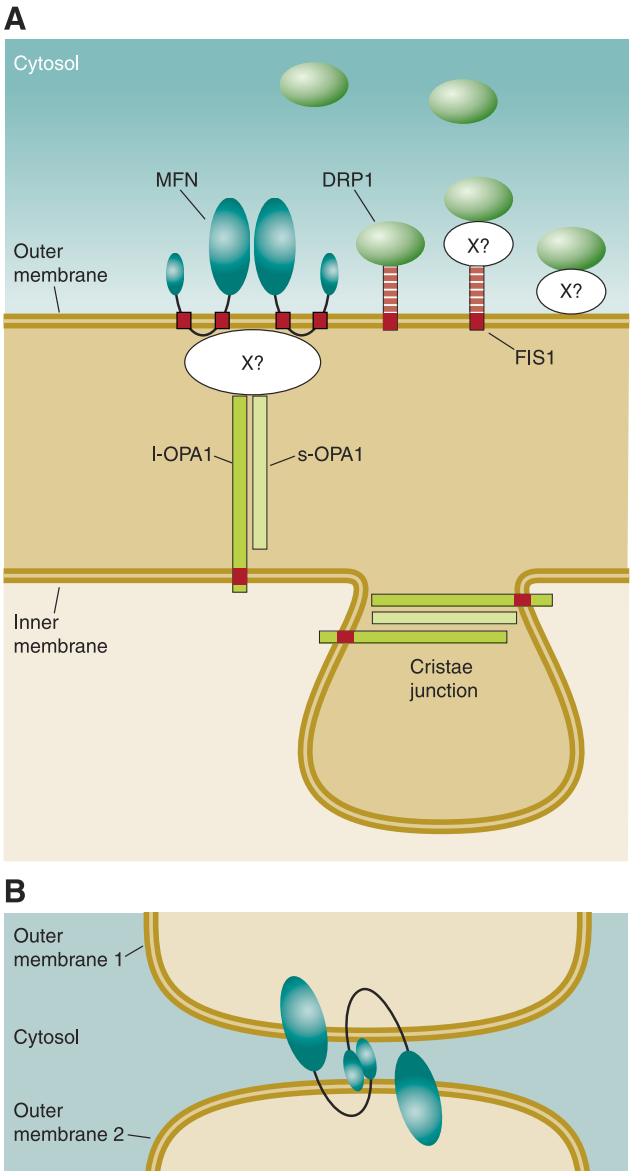


FIG. 5. Topology of the proteins involved in mammalian mitochondrial dynamics. *A*: mitofusins (Mfn) and Fis1 are located in the outer mitochondrial membrane, anchored through their transmembrane domains (bipartite in the case of Mfn). Mfns are thought to form complexes in *cis* (in the same mitochondria) through the coiled-coil 1 domain and other NH_2-terminal cytosolic regions of the protein. Dnm1l/Drp1 is mainly cytosolic and is recruited to the outer mitochondrial to mediate mitochondrial fission. This localization of Drp1 has been demonstrated to be mediated through the direct binding of Drp1 to Fis1. However, several lines of evidence show that this localization could also be through indirect binding or could be independent of Fis1. OPA1 is located in the inner membrane space in soluble forms (short forms generated by proteolytic cleavage; s-OPA1) or tightly attached to inner mitochondrial membrane (long forms, containing the transmembrane domain, l-OPA1). OPA1 is detected in the same complex as Mfns, but the nature and topology of this interaction is unknown. The requirement of a short OPA1 isoform to control the morphology of the inner mitochondrial membrane (the cristae and their junctions) has generated a model by which the interaction between s-OPA1 and l-OPA1 can control this morphology. *B*: structural data demonstrated the antiparallel interaction of the COOH-terminal cytosolic coiled-coil domains of two Mfn molecules in *trans* (that is in distinct mitochondria). The interaction depicted is thought to be the first step of mitochondrial fusion (the initial tethering between two mitochondria).

controls mitochondrial morphology as its overexpression causes mitochondrial fragmentation and its repression by RNAi induces mitochondrial elongation. The coenzyme-binding domain is essential for Mib-induced mitochondrial fragmentation, which is through inhibition of Mfn1 activity and, probably, Mfn2. Like Mfn1, Mib is widely expressed in all tissues assayed; however, in contrast to Mfn2, Mib does not show a tissue-specific expression (91). Both Mfn1 and Mfn2 coimmunoprecipitate with Mib. However, it remains to be determined whether Mib interacts directly with Mfn2 or through Mfn1 interaction, as Mfns form heterodimers and coimmunoprecipitation of Mib with Mfn2 could be due to the presence of endogenous Mfn1 in this immunoprecipitated complex. To our knowledge, no other mammalian protein has been described to specifically interact with Mfn1.

B. MFN2

MFN2 was first identified by homology with *Drosophila* Fzo (51, 92, 252, 256). However, it was also identified in two independent differential display analyses, which aimed to determine genes differentially expressed in distinct pathological conditions. The first of these analyses was performed in muscle from genetically obese rats (Zucker rats) (16), in which Mfn2 was originally named mitochondrial assembly regulatory factor (MARF). The second was done in vascular smooth muscle cells (VSMCs) of rats with genetic hypertension, a condition that induces hyperproliferation of these cells. Thus, in databases, MFN2 is also referred to as MARF and hyperplasia suppressor gene (HSG) (53). Human MFN2 is significantly conserved in vertebrates (Fig. 6). Human and fish Mfn2 show 82% sequence identity (Table 2).

1. Protein domains and activity

MFN2 is an outer mitochondrial membrane protein of 757 residues in humans and has the same functional domains as MFN1 (Figs. 4 and 5) (252, 256). It has been recently reported that Mfn2 (and not Mfn1) is also present in the endoplasmic reticulum (ER) and controls ER morphology and its tethering to mitochondria (72). The COOH-terminal part of the protein, which contains the transmembrane domain (residues 615-648 aa) and the coiled-coil 2

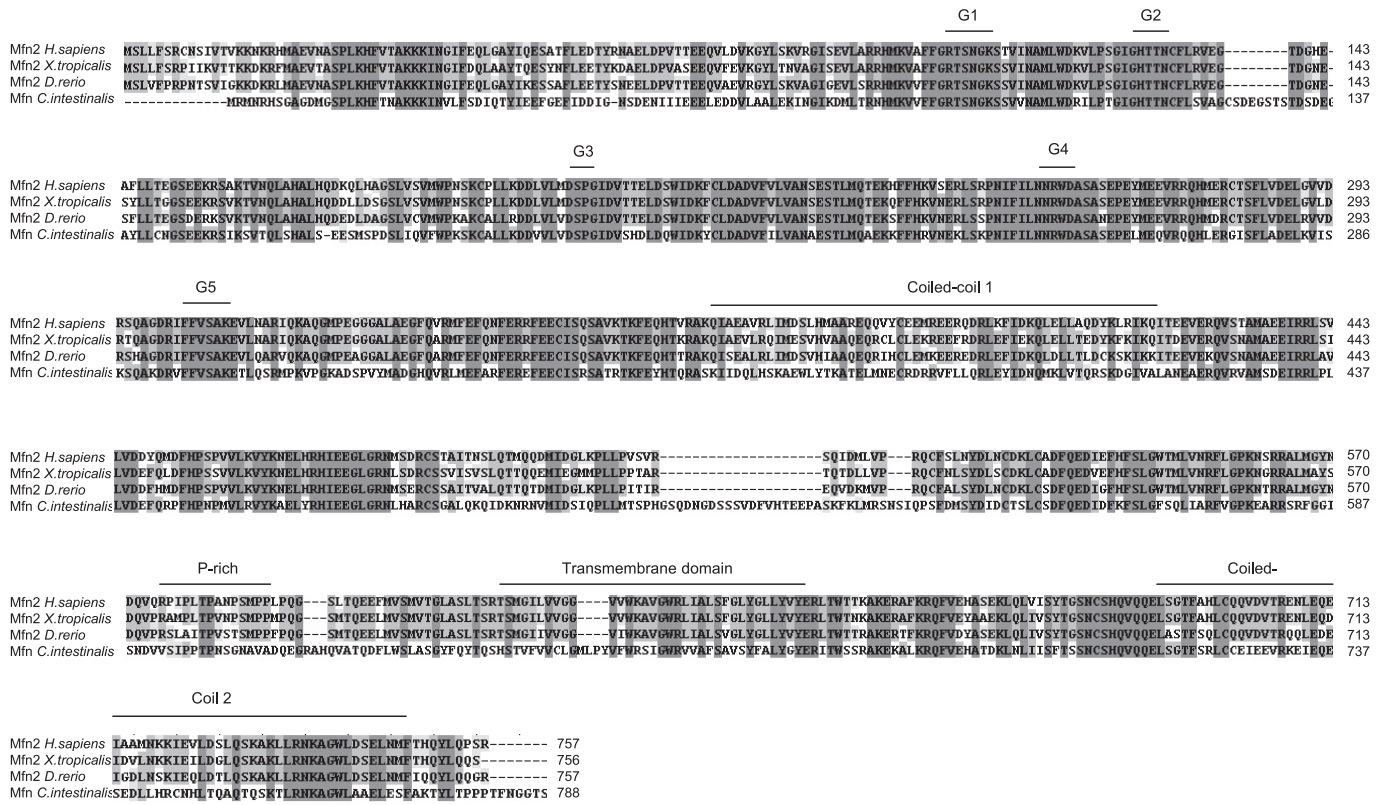


FIG. 6. Alignment of Mfn2 sequences from several chordate species and the ancient chordate *Ciona intestinalis*. Alignments were performed with Clustal W on-line software. Residues are shown in intensities of gray in function of their identity through *Homo sapiens*, *Danio rerio* (zebrafish), *Xenopus tropicalis* (frog), and *Ciona intestinalis* (prochordate). Dark gray shows the residues found in all the species analyzed, and light gray shows the residues found only in some of them. The human MFN2 protein domains are shown. Sequences used are as follows: MFN2 *H. sapiens* (NP055689), Mfn2 *D. rerio* (AP19405), Mfn2 *X. tropicalis* (CAJ82624), and Mfn *C. intestinalis* (XP_002126852).

domain (residues 693-747 aa), is also responsible for MFN2 mitochondrial localization (Fig. 5) (252, 256). More precisely, the deletion of the 648-757 region and its substitution by five amino acids (IYFFT) is sufficient to localize MFN2 only in the ER (252). This dependence of subcellular localization or targeting on the sequence flanking the transmembrane domain is similar to that reported for tail-anchored proteins, although MFN2 is an atypical tail-anchored protein as it exposes both NH_2 and COOH termini to the cytosol (252).

Generally, like MFN1, MFN2 shows a dispersed distribution throughout the mitochondrial network (51, 218, 236, 252, 256). The expression of myc-tagged or fluorescent proteins fused to MFN2 have been detected at specific mitochondrial regions or tips (mitochondrial scission sites) (139, 159, 160). The reasons for these differences in localization are currently unknown.

Protease digestion experiments of isolated mitochondria demonstrate that MFN2 is an integral outer mitochondrial membrane protein with both NH_2- and COOH-terminal parts exposed to the cytosol (252). Immunolocalization and immunoprecipitation experiments of deleted mouse Mfn2 forms show that the NH_2- and COOH-terminal parts of Mfn2 interact (NH_2-terminal Mfn2 Δ471-757 aa and COOH-terminal form Mfn2 Δ1-647 aa), thereby suggesting the formation of Mfn2 *cis* oligomers (or oligomers formed in the same mitochondria) (132, 252). These *cis* complexes are postulated to form through the coiled-coil or heptad-repeat domains (252) (Fig. 5*A*). However, the GTP-binding domain and GTPase activity, together with other conserved regions of Mfn2 protein, make a significant contribution to this NH_2- and COOH-terminal interaction, which is even greater than the contribution of coiled-coil domain 1 (366-422 aa) (132). This is shown by the decrease in the interaction and function of these Mfn2-deleted forms when a mutation is introduced in the GTPase domain of the NH_2-terminal form of Mfn2 (T130A) or when the conserved regions of the NH_2-terminal are deleted (Δ270-460 or Δ1-96 aa). These *cis* interactions could explain the partial mitochondrial localization of a human soluble MFN2 truncated form (from aa 1 to 602), which modulates mitochondrial activity without changing mitochondrial morphology (236).

GTPase activity is crucial for MFN2-mediated mitochondrial fusion (51, 92), but not for the perinuclear mitochondrial aggregates caused by MFN2 overexpression, similarly to what has been reported for MFN1 (139, 252, 255, 256). This aggregation mechanism can be explained by the same as that described for Mfn1, namely, the tethering mediated by the coiled-coil 2 domain, also present in MFN2 (693-747 aa) (175, 255). In addition, the same defect on mitochondrial mobility observed in *Mfn1* −/− cells is present in *Mfn2* −/− cells (51). Thus it could be hypothesized that deregulated mobility caused by MFN2 overexpression also explains perinuclear mitochondrial clustering. Like MFN1, the GTPase domain (103-309 aa) of MFN2 also contains five G motifs or boxes (Figs. 4 and 6). These boxes are G1 (103-110 aa), G2 (128-133 aa), G3 (199-203 aa), G4 (257-262 aa), and G5 (303-309 aa). Their specific functions in GTP hydrolysis are discussed in section II*A* (34, 35). The GTP hydrolysis activity and capacity of nucleotide exchange of Mfn2 is low, and Mfn2 binding or affinity for GTP is higher than that shown by Mfn1 (144, 216). Furthermore, MFN2 forms carrying mutations that enhance its affinity for GTP (such as G1 motif multiple mutation GRTSNGKS to GAVGVGKS, with the conserved G1 mutated residues underlined) activate mitochondrial fusion (216) and mutations that inactivate the GTPase domain (such as K109T) block this process (51, 252, 256).

As the NH_2- and COOH-terminal parts of MFN2 are exposed to the cytosol, a small region of the protein (627-632 aa) presumably faces the intermembrane space and splits the transmembrane domain in two parts (Fig. 5). Interestingly, the MFN2 mutant W631P (W631 is a highly conserved residue facing the intermembrane space, see Fig. 6) acts as an inhibitor of mitochondrial fusion (216). The inhibition exerted by this W631P mutant could also be explained by recent data showing that MFN1, MFN2 (both outer mitochondrial membrane proteins), and OPA1 (mitochondrial fusion protein located in the inner and intermembrane space of mitochondria) interact, as they were all detected in the same immunoprecipitation complex (119). Therefore, this W631P MFN2 mutant could hinder the formation of this MFN-OPA1 protein complex, thereby inhibiting mitochondrial fusion. In this regard, previous work done in yeast identified Ugo1, a protein localized in the intermembrane space that interacted with both Fzo (yeast Mfn ortholog) and Mgm1 (yeast OPA1 ortholog) (263). However, no mammalian orthologs of Ugo1 or proteins with this function have been described to date. Despite the lack of Mfn2 requirement for the mitochondrial fusion activity mediated by Opa1 mouse isoform 8 in MEFs (59), the previously mentioned data suggest a potential role of MFN2 in the MFN1/OPA1 complex in human cells. Whether this lack of Mfn2 requirement is a specific feature of the Opa1 mouse isoform 8 and/or MEFs or whether it is shared with the other OPA1 isoforms or cell types requires further study.

There are also a few lines of evidence of the posttranscriptional and posttranslational regulation of Mfn2. One example is in skeletal muscle of *Mfn2* +/− mice; in this tissue Mfn2 mRNA levels are 50% lower than in wild-type mice, and this decrease is not accompanied by changes in the protein levels (278). In addition, some evidence indicates that both Mfn1 and Mfn2 are regulated by the proteasome system, as inhibitors of this system increase the protein expression of these two Mfns (160). Therefore, Mfn1 and Mfn2 may also be ubiquitinated, as described for their yeast ortholog Fzo1 (62, 90, 160, 217).

Further studies are required to establish the relevance of these posttranslational events, their regulation, and molecular mechanisms.

2. Physiological role of MFN2 activity

MFN2 has pleiotropic roles, as shown by the relevance of its activity in distinct key cellular functions such as oxidative metabolism, cell cycle, cell death, and mitochondrial axonal transport. In fact, these additional roles (which may be mediated or not through its mitochondrial fusion activity) may explain the specific involvement reported for MFN2 but not for MFN1 in pathology. Therefore, the additional roles of MFN2 may account for the more specific tissue expression pattern of MFN2 compared with MFN1 (16, 255). Of note, mutations in the *MFN2* cause the autosomal dominant neurodegenerative disease Charcot-Marie-Tooth type 2A (336). Given the variety of functions attributed to MFN2, they will be discussed in a separate section.

3. Partners and modulators

To date, distinct MFN2 partners have been identified, namely, the protooncogenic GTPase p21 Ras (with a relatively stable association with Mfn2, as it coimmunoprecipitates in synchronized and unsynchronized cells), the mitochondrial ubiquitin-ligase membrane-associated ring CH (MARCH)-V (coimmunoprecipitated in HeLa cells overexpressing MARCH-V), the stomatin-like protein 2 (STOML2, previously identified as a component of erythrocyte cytoskeleton and interacts with endogenous MFN2 under basal conditions in HeLa cells), and the apoptosis-related proteins Bak, BCLX, and BCL2 (53, 77, 122, 214). Interestingly, immunoprecipitation studies show that MARCH-V, STOML2, BCLX, and BCL2 interact with MFN2 but not with MFN1 (77, 122, 214). In contrast, Bak interacts with both Mfn1 and Mfn2, although during apoptosis induction by treatment with azide, Bak disassociates from Mfn2 and increases its interaction with Mfn1 (39). In the case of BCL2 and BCLX, coimmunoprecipitation was detected in HEK293 overexpressing BCL2 or BCLX together with Myc-tagged Mfn2 (77). These studies therefore demonstrate that Mfn2 is present in several functional complexes in which Mfn1 is absent; this observation implies that Mfn2 is specifically regulated or modulated by other cell components or processes that do not require Mfn1 interaction or modulation. In addition, MARCH-V ubiquitinates and controls the protein levels of a component of the mitochondrial fission machinery (DRP1, discussed later) but does not ubiquitinate MFN2. Thus MARCH-V provides a common regulator of mitochondrial fusion and fission through diverse mechanisms (214).

To gain further insight into the biological function of these interactions, mapping studies showing the Mfn1 and Mfn2 domains that bind to these partners would be of great assistance.

C. OPA1

Linkage analyses identified OPA1 as the major chromosomic locus associated with autosomal dominant optic atrophy (ADOA; MIM165500) in chromosome 3 (more precisely, between 3q28-q29 regions) (87). Several years later, the gene within this locus whose mutations were responsible for ADOA neurodegenerative disease was found. This gene was also named *OPA1*, which encodes a dynamin-related GTPase of dispersed distribution throughout mitochondria that regulates mitochondrial shape (76). In parallel to this finding, *OPA1* was also identified by homology with its yeast orthologs, namely, the previously described mitochondrial shaping proteins Mgm1 (*Saccharomyces cerevisae*) and Msp1 (*Schizosaccharomyces pombe*) (3). OPA1 was demonstrated to control both mitochondrial fusion and cristae morphology (59, 116, 145, 223, 225, 227, 259). Although it is expressed in all the tissues assayed, OPA1 shows a specific tissue expression pattern, with the highest expression in the retina, brain, testis, liver, heart, skeletal muscle, and pancreas (3, 76). Conservation of OPA1 is high in vertebrates (Fig. 7), and *Danio rerio* and human OPA1 share 79% sequence identity (Table 2).

1. Protein domains and activity

OPA1 protein is localized in the mitochondrial intermembrane space in soluble forms or is tightly attached to the inner mitochondrial membrane (1, 3, 76) (Fig. 5*A*). A 93-kDa OPA1 isoform has been detected in fractions enriched in outer mitochondrial membranes from HeLa cells. This observation may be explained by soluble OPA1 interacting with integral outer mitochondrial transmembrane proteins (259). OPA1 contains an NH_2-terminal mitochondrial import sequence (MIS; in the first 150 residues), characterized by enrichment in positively charged amino acids, which confers the mitochondrial localization of OPA1 (Fig. 4). This particular region also displays three putative cleavage sites for the mitochondrial processing peptidase (MPP), which removes MIS (1-87 aa, through cleavage in the second site, residues R-X-L/F) upon import into mitochondria (1, 3, 76). After MIS, there is a putative transmembrane domain (exon 1 and 2, within the region from 97-113 aa, see Fig. 4) that is predicted to anchor or associate OPA1 forms in mitochondrial membranes (145, 225, 226, 259). This transmembrane domain may functionally act like the Pleckstrin homology domain (PH), which allows the localization of dynamins to membranes (interestingly PH is also absent in both Mfns) (Fig. 4).

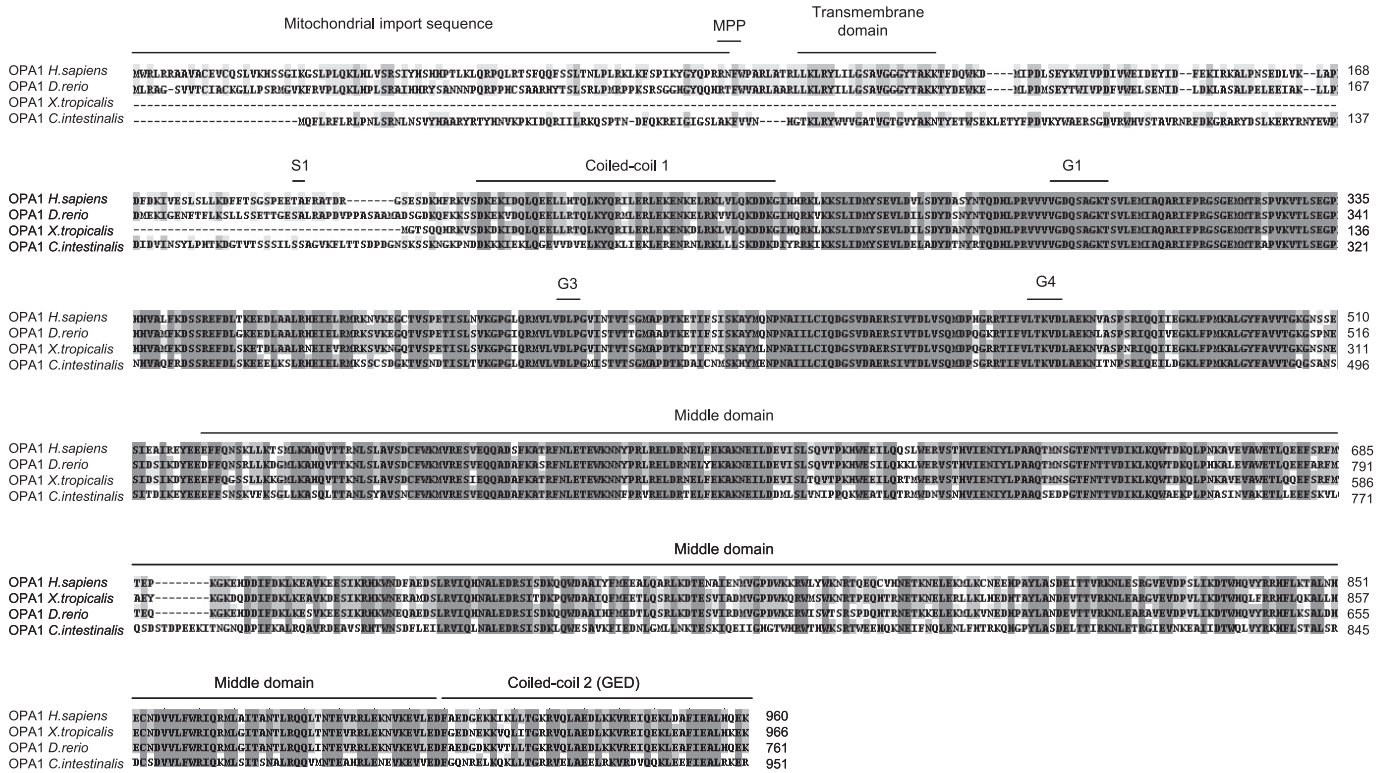


FIG. 7. Alignment of Opa1 isoform 1 sequences from several chordate species and the ancient chordate *Ciona intestinalis*. Alignments were performed with Clustal W on-line software. Residues are shown in intensities of gray in function of their identity through *Homo sapiens*, *Danio rerio* (zebrafish), *Xenopus tropicalis* (frog), and *Ciona intestinalis* (prochordate). Dark gray shows the residues found in all the species analyzed, and light gray shows the residues found only in some of them. The human OPA1 isoform 1 protein domains and cleavage sites are shown. Sequences used are as follows: OPA1 isoform 1 *H. sapiens* (NP_056375.1), Opa1 *D. rerio* (NP_001007299.1), Opa1 *X. tropicalis* (NP_001120510), and Opa1 *C. intestinalis* (XP_002131771.1).

The first OPA1 (NH_2-terminal) coiled-coil is located after the transmembrane domain (210-254 aa in human isoform 1, see Fig. 4). This region may functionally substitute the proline-rich domain found in dynamins and is basically involved in protein-protein interactions. In addition, this domain is COOH-terminal of the region that is alternatively spliced (exons 4, 4b and 5b, 149-208 aa), which implies that the topology of this coiled-coil and of all the OPA1 domains discussed from here on changes depending on the isoform (see Fig. 4). OPA1 has a second coiled-coil domain that is COOH-terminal (895-960 aa in human isoform 1), found after the GTPase and the middle domains (see Fig. 4) and thought to be the GTPase effector domain (GED or assembly domain). The GED domain is involved in the oligomerization and activation of dynamins (243). In fact, both coiled-coil domains could be responsible for the formation of the homotypic complexes between distinct OPA1 molecules. This notion is supported by the observation of self-assembly between the coiled-coil domains of Opa1 synthesized in vitro (1). Interestingly, the NH_2-terminal coiled-coil domain does not strongly interact with the COOH-terminal coiled-coil domain (1). However, further work is required to confirm these coiled-coil interactions in the context of the entire protein. These OPA1 oligomers could explain the requirement of short Opa1 isoforms (of lower molecular weight) by long Opa1 isoforms (of higher molecular weight) to mediate mitochondrial elongation and fusion in Opa1 null cells (which is discussed in sect. II*C3*) (277), as similarly reported for the yeast ortholog Mgm1 (128). Also, this GED domain could be responsible for the interaction of OPA1 with the outer mitochondrial membrane proteins MFN1 and MFN2 (Fig. 5*A*).

The GTPase domain of OPA1 (exons 7-15, 280-520 aa in human isoform 1) was described to contain 3 G boxes or motifs (the function of each motif is explained in sect. II*A*), which are the following: G1 **G**DQSA**GKT** (295-302 aa), G3 **D**LP**G** (398-401 aa), and G4 T**K**V**D** (467-470 aa) (the conserved residues are shown in bold, see Figs. 4 and 7) (3). This GTPase domain is crucial for the activity of OPA1. In this regard, fibroblasts with mutations in this domain (G300E, within G1 motif) show fragmented mitochondrial morphology, thereby demonstrating the relevance of this domain for OPA1 fusion activity (227). The middle domain is required for the assembly of high-order structures of conventional dynamins (245). Although the specific role of the OPA1 middle domain (521-894 aa in human isoform 1) remains to be determined, one may

postulate that OPA1 also oligomerizes through this domain and that this domain regulates OPA1 activity.

2. Regulation by alternative splicing

OPA1 has a marked and complex posttranscriptional regulation. In humans, it shows eight ubiquitously expressed mRNA isoforms formed by alternative splicing of exons 4, 4b, and 5b, which are located after the transmembrane domain and before the NH_2-terminal coiled-coil domain (Fig. 4) (75, 224). In mice, there are four ubiquitously expressed mRNA isoforms, also formed by alternative splicing, but only of exons 4b and 5b (1). Isoform 1 (contains only exon 4) and isoform 7 (contains exon 4 and 5b) are the dominantly expressed forms in human and mouse cells (1, 75, 145, 224). One study has shown that OPA1 isoforms containing the evolutionary conserved exon 4 (for instance, isoform 1) modulate mitochondrial fusion activity and maintain the mitochondrial membrane potential, whereas the alternate splice isoforms containing the vertebrate-specific exons 4b and 5b control cytochrome *c* release from mitochondria, and this represents a regulatory step of apoptosis specific of vertebrates (224).

3. Regulation of OPA1 by proteolysis

To make the picture more complex, OPA1 is also regulated by proteolytic cleavage, which decreases the amount of long OPA1 isoforms and increases the amount of the short ones. In a Western blot analysis, human or mouse OPA1 is detected as five distinct bands between 100 and 80 kDa. The two bands showing a higher molecular mass (known as long isoforms) are thought to be a mixture of the translation of alternative splicing mRNA isoforms 1, 2, 4, and 7 (Fig. 4). The three bands showing a lower molecular mass are considered the products of proteolysis of the long isoforms and also the product of translation of splice isoforms 3, 5, 6, and 8 (83, 145, 277) (Fig. 4). The isoforms 1, 2, 4, and 7 have the capacity to restore defective mitochondrial fusion in *Opa1* −/− cells, whereas forms 3, 5, 6, and 8 do not (277).

Both the induction of apoptosis and the specific dissipation of mitochondrial membrane potential induce the cleavage of OPA1 (to short isoforms but also to cause complete degradation of long isoforms) and a fragmentation of the mitochondrial network (12, 82, 277). Of note, one study has shown that the dissipation of the mitochondrial membrane potential does not ablate the interaction of OPA1 with MFN1/MFN2 (119). This observation suggests a decrease in the mitochondrial fusion activity of OPA1 within this complex. Importantly, the recovery of mitochondrial membrane potential induces a restoration of the mitochondrial length or fusion together with reexpression of the long (high-molecular-mass) OPA1 isoforms. These data, among others, suggest that long isoforms are crucial for OPA1-mediated mitochondrial fusion and that short isoforms are unable to fuse mitochondria (82). However, the latter isoforms are required by long isoforms to mediate fusion (277), in a similar manner to what has been described for the OPA1 yeast ortholog Mgm1 (128). This is shown by the incapacity of a mutant long OPA1 isoform 1 (a noncleavable form) to induce mitochondrial fusion in *Opa1* −/− cells (277). Furthermore, the short isoforms generated by transcription of mRNA isoforms 3 and 5 restore the capacity of this noncleavable isoform to elongate mitochondria (277).

Two of the cleavage sites in the primary sequence of OPA1 have been identified. The sites identified are named S1 and S2 (145, 277) and are located in exons 5 (S1) and 5b (S2) of the OPA1 open reading frame (Fig. 4). All the OPA1 isoforms contain the S1 site, whereas the S2 site is present only in isoforms 4, 6, 7, and 8. Therefore, proteolysis in S1 and/or S2 will generate short OPA1 isoforms, without the putative transmembrane domain (Figs. 4 and 5*A*). In this context, the loss of mitochondrial membrane potential induces OPA1 cleavage through S1, but not S2, and other still unknown additional sites (responsible for complete degradation). Interestingly, the proteases responsible for cleavage in S1 remain to be determined (119, 277). However, 1,10-phenanthroline, a metal chelator, blocks the cleavage induced by the loss of mitochondrial membrane potential, thereby suggesting the involvement of metalloproteases (22, 82, 115, 119, 145, 277).

Three types of mammalian proteases that cleave OPA1 to varying extents have been identified to date: the m-AAA proteases (m, matrix of mitochondria oriented; homo- or heteromeric complexes containing paraplegin and/or Afg3L1 and Afg3L2 subunits), the i-AAA protease Yme1L (I, intermembrane space oriented), and the presenilin-associated rhomboid-like protease (PARL) (60, 83, 101, 115, 119, 145, 277). However, these three types of enzymes have not been demonstrated to explain the whole OPA1 cleavage and complete degradation of long isoforms triggered by chemically induced loss of mitochondrial membrane potential (82, 83, 277). Furthermore, the precise contribution of these proteases to OPA1 cleavage and release to the cytosol induced by certain apoptotic stimuli has not been established.

The i-AAA metalloprotease Yme1l is responsible for cleavage in S2 under basal conditions, although this is not the only site of action of this protease (119, 277). Studies of Yme1l loss-of-function have shown that part, but not all, of the OPA1 cleavage induced by the dissipation of mitochondrial membrane potential is explained by the activity of this protease (115, 119, 277).

Regarding the m-AAA metalloproteases, paraplegin/*Spg7* alone is not a major regulator of OPA1 cleavage under basal conditions or under dissipation of mitochondrial membrane potential, as the lack of paraplegin does

not affect OPA1 processing (83, 115, 145). In contrast, two subunits of m-AAA, namely, Afg3L1 and Afg3L2, regulate basal OPA1 processing and can also form heterotypic complexes with paraplegin that are also active (83). Thus it would be of interest to unravel whether AFG3L1 and AFG3L2 are the main subunits responsible for CCCP- or apoptosis-induced OPA1 cleavage by using *Afg3L1* and *Afg3L2* double KO cells, as siRNA approaches do not allow conclusions to be drawn (115). Moreover, these two particular m-AAA subunits are differentially expressed in tissues (83), which makes them interesting candidates to study the particularities of mitochondrial dynamics in each tissue. In addition, it would be of great interest to determine the exact sites of action of AFG3L1 and AFG3L2 in the OPA1 sequence.

PARL is the mammalian ortholog of yeast Pcp1, the inner-mitochondrial membrane protease that cleaves and regulates Mgm1 activity (the yeast ortholog of OPA1). Thus it was the natural candidate to regulate mammalian OPA1 processing. Indeed, PARL regulation by phosphorylation and cleavage of its β-NH_2-terminal region modulates mitochondrial morphology (149). However, PARL does not explain the whole OPA1 processing under basal conditions or triggered by the loss of mitochondrial membrane potential in mammalian cells (12, 22, 60, 101). To date, the main identified role for PARL is the generation of a soluble Opa1 isoform detected in the intermembrane space (IMS), which represents 4% of total IMS Opa1 in mitochondria (60, 101). This soluble Opa1 isoform controls the shape of mitochondrial cristae, independently of its role in mitochondrial fusion (see Fig. 5*A*). This Parl-mediated processing of Opa1 was demonstrated by two key observations: *1*) the Opa1-soluble isoform is dramatically reduced in *Parl* −/− MEFs, and *2*) an inactivated version of *Parl* (mutant H335G) does not restore the production of soluble Opa1 in *Parl* −/− MEFs (60, 101). Additionally, PARL coimmunoprecipitates with OPA1 (60). However, whether the primary sequence of OPA1 holds a PARL cleavage site remains to be determined. Thus further data are required to demonstrate how this soluble isoform is generated.

These lines of evidence suggest that, in spite of the significant findings and advances made in OPA1 processing, additional enzymes or molecular mechanisms remain to be discovered. In this regard, a recent study demonstrates a marked increase in Opa1 cleavage by the lack of Prohibitin 2 (Phb2), a highly conserved protein of poorly understood function, thought to act as a scaffold by forming complexes with several proteins. Of note, the protease or the molecular mechanism responsible for the increase in Opa1 cleavage caused by the lack of prohibitin 2 (Phb2) remains to be determined (206).

In summary, all these studies demonstrate that OPA1 activity has a complex posttranslational regulation, which allows rapid changes in mitochondrial morphology, activity, and apoptosis induction in response to distinct stimuli. Given the complex formed by OPA1 and MFNs (119), the tight and rapid OPA1 regulation and the observation that mitochondrial outer and inner membrane fuse in separate but coordinated steps, we hypothesize that OPA1-mediated mitochondrial fusion and its regulation are the bottle-neck of the entire mitochondrial fusion process.

4. Role of OPA1 in mitochondrial fusion and physiology

The overexpression and loss-of-function studies of OPA1 demonstrate that this protein is involved in the control of mitochondrial fusion and cristae morphology. However, as reported in some of these studies, OPA1 overexpression leads to the fragmentation of the mitochondrial network, as reported in OPA1 loss-of-function studies (siRNA or KO cells) or in conditions characterized by an enhanced rate of mitochondrial fission (12, 51, 115, 223). In contrast, other studies show that the overexpression of a mouse isoform 8 (similar to human isoform 1) of Opa1 either does not affect or elongates the mitochondrial filaments in control cells (50, 59). This differential response to Opa1 could be explained by the extent of Opa1 overexpression caused by the use of distinct vectors (containing distinct promoters). Furthermore, Opa1 overexpression restores and increases mitochondrial fusion in cells with an intrinsic defect in this process (such as *Opa1* or *Mfn2* KO cells) (59, 277). However, as mentioned when summarizing the effects of MFN1, Opa1 overexpression does not restore mitochondrial fusion in *Mfn1* KO cells (59).

This paradox (fragmentation by OPA1 overexpression) could be explained by the presence of distinct endogenous OPA1 isoforms generated by alternative splicing and proteolysis (generally classified by long and short isoforms). These distinct OPA1 molecules may have the capacity to interact or oligomerize between them and, importantly, some (long isoforms) require the presence of the others (short isoforms) to mediate mitochondrial fusion (1, 277). Therefore, excessive OPA1 overexpression in some cells probably alters the proportion of the endogenous isoforms and their oligomers, thereby leading to a dominant negative effect in terms of the control of mitochondrial morphology, which could explain this mitochondrial fragmentation. Thus lower OPA1 overexpression levels (due to different promoters) could explain the lack of OPA1-induced fragmentation, or even elongation, reported in some studies.

In addition to mitochondrial fusion activity, OPA1 loss-of-function studies have shown that RNAi-mediated OPA1 repression activates apoptosis. Furthermore, they also demonstrate that a reduction in OPA1 causes aberrant cristae structures (12, 116, 223). The altered cristae morphology reported could be secondary to apoptosis

activation. However, the demonstration of a direct control of cristae morphology by OPA1 was provided. Thus the previously mentioned soluble isoform of OPA1 (generated by PARL-mediated cleavage) determines the size of mitochondrial cristae junctions by forming a complex with distinct molecules of the long OPA1 isoforms anchored in the inner membrane. This complex regulates the size of the cristae junction and controls the amount of cytochrome *c* release induced by apoptosis (60, 101, 323). Thus it is not surprising that OPA1 repression also decreases both cell growth and oxygen consumption (12, 50, 181, 223).

Furthermore, a recent study reports that the specific posttranslational regulation of OPA1-mediated mitochondrial fusion allows the control of one key step of the fate of mitochondria to be eliminated through autophagy. Damaged mitochondria normally show a decrease in mitochondrial membrane potential, which in turn activates complete degradation of OPA1 (not only the processing to short isoforms) (277). This complete degradation inhibits the fusion of these damaged mitochondria to the mitochondrial network. If these mitochondria are still damaged, they are unable to restore the mitochondrial membrane potential, which implies that they remain incapable of fusing and will then enter autophagy (302). Therefore, these data demonstrate that OPA1 is the key fusion factor for autophagy. Indeed, these data point to a novel pathogenic mechanism of the alteration of mitochondrial fusion and fission rates.

The physiological relevance of OPA1 activity has also been described at the level of a whole organism by the embryonic lethality of Opa1-ablated mice (days 8.5-10.5, depending on the model) (2, 51, 71). Moreover, heterozygous mutant Opa1 mice (which display around a 50% reduction in Opa1 protein levels) show a visual impairment similar to that described for human ADOA, thereby confirming a haploinsufficiency defect in this disease (2, 71). However, the relevance of OPA1 regulation of apoptosis or autophagy to this phenotype is not yet known. This lack of compensation in heterozygous *Opa1* mutant mice is in marked contrast to the compensation of *Mfn2* +/− heterozygous mice, which do not show any reduction in Mfn2 protein levels or any pathogenic phenotype (51, 278). These data, together with the marked regulation of OPA1 by proteolysis, demonstrate that the posttranslational regulation of MFN2 and OPA1 clearly differ, although both proteins can be found in the same complex that regulates mitochondrial fusion.

Furthermore, Opa1 and Mfn2 also show differences at the level of transcriptional regulation. The first difference is the distinct control of their expression under several physiological conditions. Thus cardiac differentiation markedly decreases Opa1 mRNA levels, whereas it increases Mfn2 mRNA levels (58). In addition, the nuclear coactivator PPARGC1B (PGC-1β), which controls basal mitochondrial biogenesis and is highly expressed in heart, stimulates mitochondrial fusion mainly through preferential control of Mfn2 expression compared with the rest of components of the mitochondrial dynamics machinery (i.e., Opa1) (189). Therefore, if these proteins control mitochondrial fusion only through the formation of the complex with Mfn1, Mfn2, and Opa1, one would expect similar inductions of their expression.

The meaning or the true extent of these specific differences of regulation between mitochondrial fusion proteins will not be fully understood until the mechanisms of coordination and the relationship between mitochondrial inner and outer membrane fusion are solved at a higher resolution level. This elucidation will allow us to determine new checkpoints of mitochondrial fusion, the specific involvement of MFN1, MFN2, or OPA1 in them, and how the cell modulates these processes. Also, a complete definition of the specific functions of each protein (those less related to mitochondrial fusion or more related, for instance, to intracellular signaling) will greatly contribute to our understanding of these differences and the meaning or physiological relevance of mitochondrial dynamics.

III. REGULATION OF MITOCHONDRIAL FISSION: PROTEINS INVOLVED IN MITOCHONDRIAL FISSION IN MAMMALIAN CELLS

Mitochondrial fission involves a crucial feature that is not present in mitochondrial fusion. This feature is that the main proteins that control mitochondrial fission also regulate peroxisomal fission (172, 173). In this regard, it is not known whether the fission of these two organelles always runs in parallel or whether they are differentially regulated. This issue is highly relevant when addressing whether the physiological consequences of modulating or regulating the components of the fission machinery are due to the modulation of mitochondrial morphology, of peroxisome dynamics, or both. The answer to these questions will also provide key clues to the biological relevance of this coincidence. The most studied proteins involved in mitochondrial fission and found also in peroxisomes are the dynamin-related protein 1/dynamin 1 like (DRP1/DNM1L) and fission protein 1 homolog (FIS1), which are located in the outer membrane in the case of mitochondria. However, whether there is specific inner membrane fission machinery remains to be established. In the case of yeast, MDM33 is the most relevant protein described to date to be involved in the inner membrane fission machinery; however, no mammalian orthologs of MDM33 have yet been identified (207). Indeed, there are only two reports that demonstrate that MTP18 is a member of this unknown inner membrane fission machinery, acting in a similar manner to MDM33 (298, 299).

A. DRP1/DNM1l/DLP1

Several laboratories identified *DNM1L* (dynamin 1-like gene) in mammalian cells as a gene related to the superfamily of dynamin proteins that mediate the constriction of nascent endocytic vesicles and secretory membrane compartments (243). Therefore, it is also known as DRP1, DYMPLE, DVLP, or DLP (142, 155, 269, 274, 326). DNM1L/DRP1 is the mitochondrial dynamics protein with the highest degree of conservation in chordates (Table 3 and Fig. 8). Drp1 was found to be expressed in all tissues assayed. However, the brain expresses a specific Drp1 isoform, generated by alternative splicing, that is absent or not detected in other tissues (274, 326). The tissues with high levels of Drp1 expression are brain, skeletal muscle, and heart. Intermediate levels of Drp1 expression are found in testis, kidney, and pancreas while low levels are detected in liver and spleen (274, 326).

In these forerunning descriptions, DRP1 (dynamin-related protein 1) was thought to be a mediator of vesicle formation required in the early secretory pathway (142). In addition, this protein was described to control ER morphology (240, 326). However, the inhibition of DRP1 activity also revealed a marked alteration of mitochondrial morphology, causing extremely long perinuclear mitochondria (240, 274). This mitochondrial phenotype led to the discovery that DRP1 controls mitochondrial division in mammalian cells and *C. elegans* (177, 273), similarly to what is reported for the yeast ortholog Dnm1p (31, 129).

The localization of DRP1 is mainly cytosolic, but a considerable part of this protein is detected in mitochondria, and more precisely is found in concrete puncta or dotted structures, some of which are located on future mitochondrial scission sites or in mitochondrial tips (Fig. 5*A*) (273). The interest in this gene increased further after the demonstration that DRP1-mediated mitochondrial fission is relevant for the induction of apoptosis, as inhibition of DRP1 decreases the susceptibility of the cell to enter programmed cell death (97). Later, DRP1 was also detected in tips or discrete regions of peroxisomes, where this protein also controls the fission of this organelle (172). Importantly, under basal conditions, the recruitment of DRP1 from the cytosol to mitochondria is dependent on dynein and microtubules (306). Furthermore, recruitment of DRP1 to mitochondria induced by inhibitors of OXPHOS and cyclosporin A is mediated by F-actin cytoskeleton and not by microtubules (73). Therefore, it is likely that the cytoskeletal components required for DRP1 recruitment differ depending on whether DRP1 catalyzes basal or induced mitochondrial fission.

Taken together, the above evidence suggests that DRP1 plays distinct roles in cell function and has a tightly regulated recruitment. Thus the future generation of Drp1-ablated mice will permit the study of the diverse physiological effects of Drp1 in numerous organelles and cellular processes. Although these mice have not been reported to date, several studies have elucidated the posttranslational regulation of DRP1, its effects on mitochondrial recruitment, and its role in apoptosis and have thus contributed to our understanding of DRP1 function in cell biology. We will briefly summarize these posttranslational modifications of DRP1 in the light of their physiological effects. Regarding more specific details on the posttranslational regulation of this protein, readers are referred to an excellent review on this topic published while we were writing this manuscript (254).

TABLE 3. *Conservation of mitochondrial fission proteins (chordates and prochordates)*

Organism and Percent Identity to *H. sapiens*	Dnm11	Fis1
R. norvegicus	98	96
M. musculus	98	96
X. laevis	91	NF
X. tropicalis	NF	75
D. rerio	90	NF
C. intestinalis	64	44

NF, not found in databases. Percentages of identity were obtained using ClustalW2 on-line software. DNM1L *H. sapiens* (NP_005681.2), Dnm1l *M. musculus* (NP_001021118.1), Dnm1l *R. norvegicus* (NP_446107.2), Dnm1l *D. rerio* (NP_957216.1), Dnm1l *X. laevis* (NP_001080183.1), Dnm1l *C. intestinalis* (XP_002130043.1). FIS1 *H. sapiens* (NP_057152.2), Fis1 *M. musculus* (NP_079838.1), Fis1 *R. norvegicus* (sp P84817 FIS1_RAT), Fis1 *X. tropicalis* (NP_001123678.1), Fis1 *C. intestinalis* (XP_002121705.1).

1. Protein domains and activity

The canonical dynamin domains present in human DRP1 (isoform 3, 699 residues) are the GTPase domain (residues 1-300), the middle domain (residues 301-508), and the GED or assembly domain (residues 599-699) (274) (see Fig. 9). There is also a less defined region, called the divergent domain (residues 509-598), where the insert of 37 amino acids in position 533 generated by alternative splicing of DRP1 mRNA is found only in brain (274, 326) (Fig. 6). The sequence of the GTPase domain was described to contain three G motifs or boxes (G1, 32-40 aa; G3, 146-150 aa; G4, 215-218 aa; function explained in sect. II*A*) (Figs. 8 and 9). Site-directed mutagenesis and chimera generation of DRP1 demonstrate the relevance of these domains in the regulation of DRP1 on mitochondrial and peroxisome fission. Indeed, the single deletion of one of these three domains (GED, the GTPase or the middle domain) blunts the mitochondrial function of this protein (239).

More precisely, and assuming that DRP1 behaves in a similar manner to its yeast ortholog Dnm1p, GTP hydrolysis is required to provide the mechanical force to con-

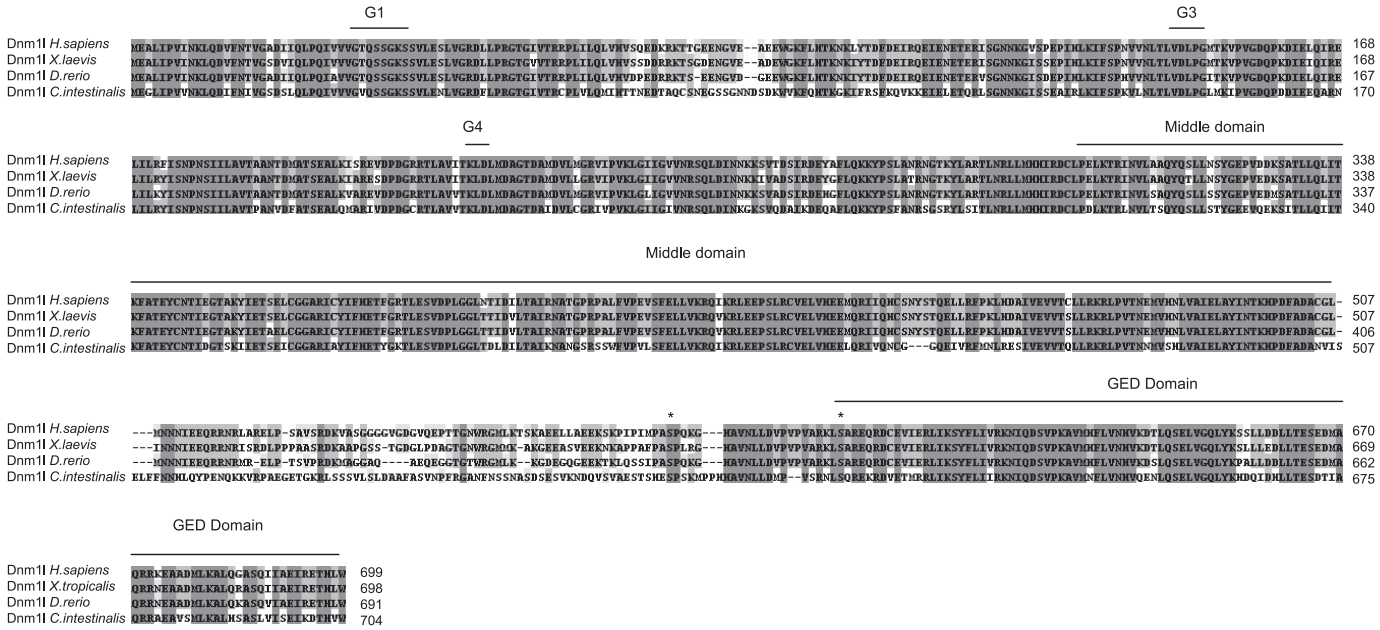


FIG. 8. Alignment of Dnm1l/Drp1 sequences from several chordate species and the ancient chordate *Ciona intestinalis*. Alignments were performed with Clustal W on-line software. Residues are shown in intensities of gray in function of their identity through *Homo sapiens*, *Danio rerio* (zebrafish), *Xenopus laevis* (frog), and *Ciona intestinalis* (prochordate). Dark gray shows the residues found in all the species analyzed and light gray the residues found only in some of them. Asterisks mark the serine residues described to be phosphorylated by distinct kinases (residues 579 and 600 in isoform 3, 616 and 637 in splice variant 1/brain isoform; see Figs. 9 and 10). The human DNM1L protein domains are shown. Sequences used DNM1L isoform 3 *H. sapiens* (NP_005681.2), Dnm1l *D. rerio* (NP_957216.1), Dnm1l *X. laevis* (NP_001080183.1), and Dnm1l *C. intestinalis* (XP_002130043.1).

strict the mitochondrial tubule by DRP1 oligomers, which surround it, thereby forming a spiral ring around the scission site (133, 143, 273, 327). In this regard, mutations that potentially blunt GTP-binding (such as K38A) generate extremely long and interconnected mitochondria by inhibiting mitochondrial division (304, 327). This DRP1 mutant (K38A) is not only inactive but also sequesters endogenous DRP1 and reduces its mitochondrial localization, thus exerting an additional dominant negative effect (148, 327). The mitochondrial phenotype induced by DRP1 K38A is the same as that observed in cells where DRP1 expression is reduced by siRNA (97, 240, 273, 274).

The function of the middle domain of DRP1 remains to be determined. This middle domain may be vital for the initial oligomerization of DRP1 required to form the proper high-assembly structures on membranes (i.e., the spiral rings). This hypothetical role for the middle domain of DRP1 has been recently demonstrated in conventional dynamins (245). Therefore, it is likely that this domain has a similar function in DRP1. In agreement with this hypothesis, the GTPase and middle domains of DRP1 also participate in DRP1 intra- and intermolecular interactions through binding to the GED (333). Structural analyses of the middle domain may be crucial to identify its activity.

The GED or assembly domain, in addition to being crucial for the regulation of GTPase activity and for the intra- and intermolecular associations (such as those reported for dynamins and the yeast DRP1 ortholog Dnm1p) (103, 333), also contains the information necessary for the mitochondrial targeting of DRP1 (239). Therefore, mutations in this domain also markedly change DRP1 activity in mitochondrial division (333).

Although DRP1 is a protein related to dynamins, some key domains of this superfamily of proteins are absent in the amino acid sequence of DRP1. These absent domains are the PH and proline-rich domains. Importantly, DRP1 does not have any transmembrane domain that functionally substitutes this PH, such as that described in OPA1 or MFNs. Thus to be located on the mitochondrial or peroxisomal membranes, DRP1 requires interaction with a protein anchored to the membrane of these organelles. The membrane protein described to functionally interact with DRP1 is FIS1 (148, 325). Moreover, FRET assays, and cross-linking and immunoprecipitation studies indicate that DRP1 binds directly to FIS1 (325).

However, several lines of evidence suggest that this is not the only mechanism by which DRP1 promotes fission. The first is that the yeast ortholog Dnm1p requires two adaptor proteins (Mdv1 and Caf4) to bind indirectly to Fis1 (114, 295). However, no orthologs of these proteins have been described to date that confirm this in mammalian cells. The second line of evidence comes from siRNA studies performed to repress FIS1 expression, in which DRP1 was still able to localize on the outer mitochondrial membrane (181). The third line is the find-

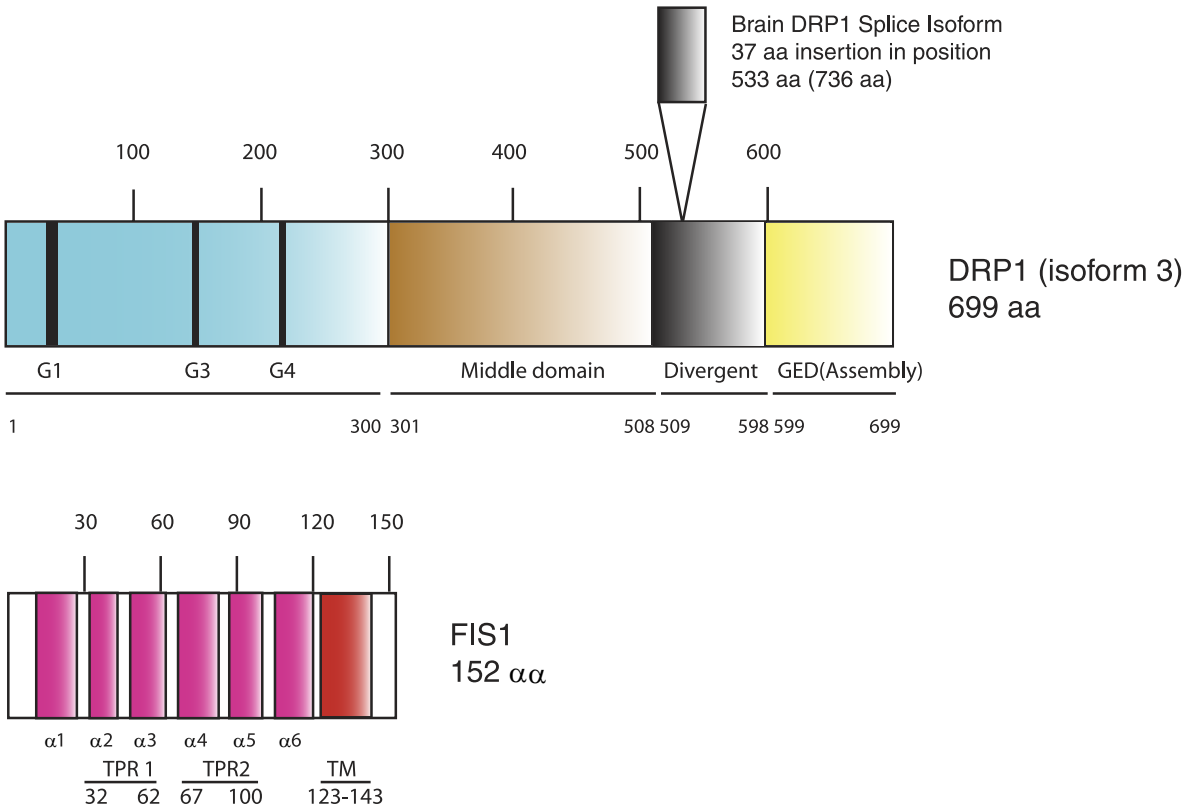


FIG. 9. Domains and motifs identified in human mitochondrial fission proteins FIS1 and DRP1/DNM1L. The length and topology in amino acids for each region and domain are shown. DRP1/DNM1L: the GTPase domains are shown in blue with the distinct G motifs shown in black bars (G1, G3, and G4). The coiled-coil region (CC) or GED (GTPase effector domain or assembly domain) is shown in yellow. The middle domain is colored in brown and the divergent region in black. The insertion of 37 amino acids found in the DRP1/DNM1L splice variant 1 (brain isoform) generated by alternative splicing is also shown and is located in the divergent region (position 533). FIS1: structural data demonstrated the presence of 6 α-helices (shown in pink) and four of them conformed two TPR motifs (tetratricopeptide repeat motifs). The transmembrane domain (TM) is shown in red.

ing that the stabilization of DRP1-binding on the mitochondrial membrane is independent of FIS1 expression levels under a number of conditions (see summary in Fig. 5*A*) (126, 316). Therefore, more experimental data are required to demonstrate the mechanistic details of the interaction between DRP1 and FIS1 and also to identify novel Drp1-associated proteins.

2. Physiological regulation of DRP1 activity

Given that DRP1 is constantly changing localization from the cytosol to the mitochondria (273, 316), most research attention has focused on the mitochondrial recruitment and the posttranslational regulation of this protein. In fact, an understanding of the regulation of DRP1 localization will explain how some cellular processes mediate mitochondrial fragmentation through this protein. Moreover, elucidation of this regulation will help us to understand why some of these processes require an enhanced rate of mitochondrial fission through the control of DRP1 activity. To date, the cellular processes reported to modulate DRP1 activity, or vice versa, are apoptosis, neuronal function, cardiac and muscle differentiation, cell cycle, the second-messenger molecule cAMP through protein kinase A (PKA) and calcium, the latter through CaMKI and the phosphatase calcineurin. The physiological processes modulated by DRP1 activity are briefly discussed in this section and in depth in the sections on mitochondrial dynamics, cell death, and cell cycle.

3. Apoptosis

Most studies addressing the regulation of DRP1 have been done in the light of the apoptotic process, as DRP1 and mitochondrial fission modulate programmed cell death (97). Moreover, during apoptosis DRP1 localizes in mitochondria punctae where MFN2 and the proapoptotic protein BAX are also present (this being the first evidence of a probable complex between mitochondrial fusion, fission, and proapoptotic proteins) (159). The first proteins described to stimulate DRP1 recruitment to mitochondria induced by apoptosis are as follows: the cleavage product of the integral ER protein BAP31 (named p20, which maintains the localization in the ER membrane),

deafness dystonia protein (DDP/TIMM8a), and BIK (13, 38, 109).

p20-BAP31 is generated by caspase-8 during the early stages of apoptosis and induces the release of calcium from the ER. This calcium release causes an increase in calcium uptake by mitochondria together with an enhancement of the recruitment of DRP1 to these organelles, thereby stimulating mitochondrial fission. Furthermore, the overexpression of the dominant negative DRP1 mutant (K38A) greatly inhibits the cytochrome *c* release caused by the overexpression of p20-BAP31. This finding thus demonstrates the relevance of mitochondrial dynamics and DRP1 also for the progression of apoptosis initiated by ER factors (38). Of note and in marked contrast, one report describes the antiapoptotic effects of DRP1 in programmed cell death mediated by calcium, as DRP1-induced mitochondrial scission blocks the intramitochondrial calcium waves required for apoptosis progression. Importantly, this was the first and only description reported to date of DRP1-mediated resistance to programmed cell death (289). Taken together, these reports suggest that, also in calcium-mediated apoptosis, DRP1 has independent sites of action that could explain the DRP1-mediated protection of calcium-induced apoptosis under certain conditions and the enhancement of apoptosis induced by the calcium release from the ER (p20-BAP31).

The second molecule, DDP/TIMM8a, is a protein of the mitochondrial intermembrane space. Like other apoptogenic proteins, DDP/TIMM8a is released to the cytosol upon permeabilization of the outer mitochondrial membrane caused by activation of BAX/BAK by programmed cell death. DDP/TIMM8a interacts with the COOH-terminal region of DRP1 (middle and GED regions) and increases the recruitment of DRP1 to mitochondria, which in turn activates mitochondrial fragmentation (13). This study confirmed that the permeabilization of the outer membrane occurs before mitochondrial fission and DRP1 recruitment. In agreement with this, it also demonstrated that the retention of cytochrome *c* caused by the inhibition of DRP1-mediated mitochondrial fission is not due to an inhibition of the permeabilization of the outer membrane, as this and other mitochondrial proapoptotic factors are released under these conditions (13).

Apoptosis, specifically the proapoptotic proteins BAX/BAK, also enhances the stabilization of DRP1-binding on the outer mitochondrial membrane through DRP1 sumoylation (126, 316, 337). Therefore, this is an additional mechanism to explain the increase in mitochondrial DRP1 levels caused by apoptosis, which differs from the increase in DRP1 recruitment to mitochondria through DDP/TIMM8a or p20-BAP31. Moreover, this sumoylated DRP1 is proposed to contribute to stimulating apoptosis through a specific role in the remodeling of mitochondrial cristae and not through the fragmentation of the mitochondrial network (109, 316). This DRP1-mediated cristae remodeling is induced by the proapoptotic ER protein BIK, which also stimulates DRP1 recruitment to the outer mitochondrial membrane (109). However, the mechanism by which DRP1, located on the outer mitochondrial membrane, contributes to the remodeling of mitochondrial cristae during apoptosis and independently of mitochondrial fragmentation is largely unknown (109, 316).

4. Neuronal function and differentiation

The other process in which DRP1 has a relevant role is neuronal function and differentiation. Drp1 expression is markedly increased upon differentiation of primary cultured cortical neurons, together with the mentioned decrease in Mfn1 expression (49). In agreement with these data, the increase in Drp1 expression in differentiated neurons elevates the number of dendrites in primary cultured cells, whereas an increase in mitochondrial fusion or inhibition of mitochondrial fission decreases this number (188). These data suggest that a higher rate of mitochondrial fission is required for the specific function or plasticity of differentiated neurons. In this regard, it has been recently described that fibroblasts from patients with Alzheimer's disease show elongated mitochondria and a marked decrease in DRP1 expression. In addition, the same study indicates that β-amyloid accumulation triggers this reduction in DRP1 expression (314).

Moreover, there is also a link between Parkinson's disease and mitochondrial morphology. Mutational analysis revealed that PINK1 (PTEN-induced kinase 1) is involved in a recessive familial form of Parkinson's disease. PINK1 is a mitochondrial-located serine/threonine kinase and also an ubiquitin ligase that acts upstream of parkin (which is an E3-protein ubiquitin ligase). The loss-of-function of Pink1 causes an elongation of mitochondrial filaments, and this elongation may lead to neuronal alteration (241, 324). Although no physical interaction has been detected between Pink1 and mitochondrial dynamics proteins, it acts through Drp1 and Fis1 to increase mitochondrial fission (324). This is demonstrated by the loss of mitochondrial fragmentation induced by Pink1 overexpression in cells in which Drp1 or Fis1 activity is inhibited. Thus these data suggest that elongated mitochondria by abnormal Pink1 modulation of Drp1 or Fis1 contribute to the impairment of the specific neuronal function. More data are required to elucidate the molecular mechanisms by which Pink1 modulates the activity of mitochondrial fission proteins.

5. Cardiac and muscle differentiation

Drp1 expression is diminished during the differentiation of stem cells to cardiomyocytes, which show longer mitochondrial filaments (58). Therefore, both in cardiomyocytes and muscle cells, mitochondrial filaments are

longer than in undifferentiated cells, which can be explained by the different expression levels of mitochondrial dynamics components (16, 58). Skulachev and coworkers (7, 272) proposed a biological explanation for these mitochondrial filaments in muscle and several functional studies were done. Knowledge on the specific proteins that control mitochondrial dynamics will permit to unravel, at a more mecanistic level, the biological role of these distinct morphologies in cardiac or muscle tissues.

6. Mitochondrial architecture in leukocytes

One study has demonstrated that the regulation of mitochondrial distribution is essential for the chemotaxis of leukocytes (43). Thus blockade of DRP1 activity or activation of mitochondrial fusion inhibits chemotactic migration (43). Therefore, mitochondrial fission processes mediated by DRP1 and concomitant mitochondrial relocalization are crucial in the physiology of leukocytes.

7. Modulation of DRP1 activity by phosphorylation

A number of studies have shown that Drp1 and its effects on mitochondrial dynamics are regulated by phosphorylation, thereby providing significant clues as to the intracellular signals that induce mitochondrial fission (see Fig. 10). The first report demonstrated that cyclic dependent kinase 1 (Cdk1/cyclin B) phosphorylates rat Drp1 brain isoform in serine-585 (serine-616 in human splice variant 1/brain isoform) during mitosis. This phosphorylation enhances mitochondrial fission (290), thereby allowing the proper distribution of mitochondria within daughter nascent cells. The protein phosphatase involved in the removal of serine-585 phosphate is unknown.

Another protein kinase reported to phosphorylate DRP1 brain isoform in serine-637/656 (human/rat) is PKA (48, 68). The studies on human DRP1 brain isoform demonstrated that phosphorylation in serine-637 blocks intramolecular interactions between the GED and GTPase/middle domains of DRP1, accompanied by a decrease in DRP1 GTPase activity (48). However, there are discrepancies regarding this mechanism, as the studies by Cribbs and Stack (68) reported no alteration in the GTPase activity of rat serine-656-phosphorylated Drp1. Nevertheless, these and a third report showed that this phosphorylation reduces mitochondrial fission, as also demonstrated by the overexpression of phosphomimetic mutants of DRP1 (S637/656D), which markedly elongate mitochondrial filaments (47, 48, 68). Thus PKA phosphorylation inhibits DRP1 activity, whereas Cdk1/cyclin B activates it (Fig. 10).

Calcineurin is the phosphatase responsible for the dephosphorylation of Ser-637, as it coimmunoprecipitates with DRP1 and treatment with specific inhibitors of this phosphatase blocks the calcium-dependent dephosphorylation of DRP1 in conditions of chemically induced calcium release (47, 68).

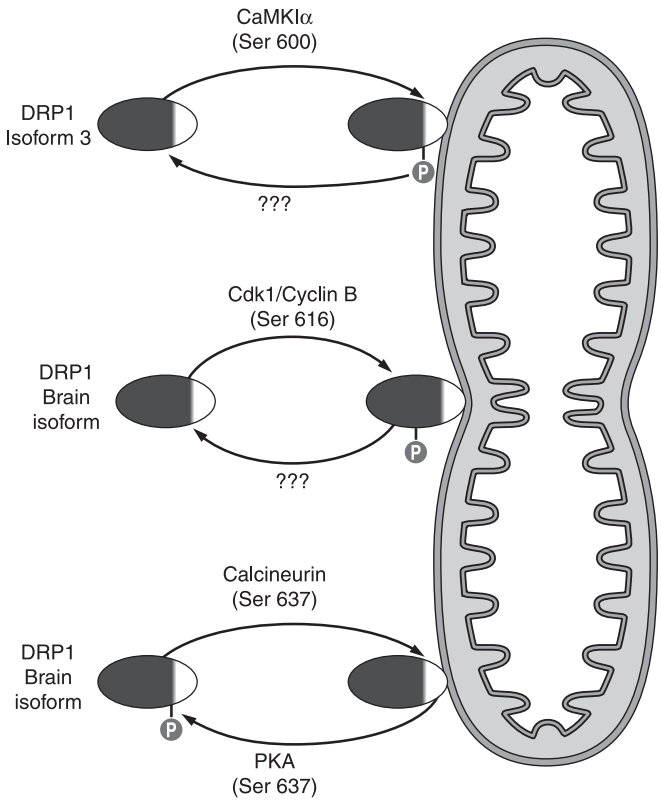


FIG. 10. Model of Drp1/Dnm1l regulation by phosphorylation. There are three kinases described to date that phosphorylate Drp1/Dnm1l (CaMKI, PKA, and Cdk1/Cyclin B) in different serine residues located in the divergent domain and/or near the GED domain (see Fig. 8). The topology of these serine residues differs in *Rattus norvegicus* and *Homo sapiens* and in brain isoform/splice variant 1 and isoform 3. Therefore, the initial description of phosphorylation in serine-585 of rat Drp1 corresponds to serine-616 of human DRP1 splice variant 1/brain isoform. Of note, whereas phosphorylation in serine-616 of splice variant 1/brain isoform and serine-600 of isoform 3 enhances DRP1 mitochondrial fission activity, phosphorylation in serine-637 of splice variant 1/brain isoform diminishes it, possibly because of a decrease in intramolecular interactions of DRP1 splice variant 1/brain isoform, which could hypothetically affect mitochondrial recruitment or stabilization. In the case of serine-600 of DRP1 isoform 3 (although it is the same residue as serine-637 of splice variant 1), its phosphorylation by CaMKIα (calcium/calmodulin-dependent kinase Iα) increases DRP1 affinity for FIS1 and its mitochondrial recruitment and/or stabilization. Although phosphorylation by Cdk1/cyclin B (cyclin-dependent kinase 1/cyclin B) of serine-616 (splice variant 1) increases mitochondrial fission, possibly as a result of greater recruitment and/or stabilization of Drp1 to mitochondria, the mechanism of Drp1 activation by serine-616 phosphorylation remains to be determined. The phosphatases responsible for dephosphorylation of serine-600 and serine-616 residues are unknown. In the case of serine-637, calcineurin is the phosphatase responsible for its dephosphorylation.

A recent report demonstrates that DRP1 isoform 3 is also phosphorylated in serine-600 (same residue that serine-637 DRP1 brain isoform, as isoform 3 does not contain the 37 amino acids insertion of brain isoform, see Fig. 9) by the Ca^{2+}/calmodulin-dependent protein kinase Iα (CaMKIα) (125). This DRP1 phosphorylation is observed upon the induction of a calcium influx through

voltage-dependent calcium channels activated by high potassium extracellular levels. In contrast, phosphorylation of serine-600-DRP1 isoform 3 changes its intracellular distribution and increases its binding to mitochondria, both in HeLa cells and neurons (125). One of the mechanisms responsible for the greater presence of serine-600-phosphorylated DRP1 on the outer mitochondrial membrane could be explained by the higher affinity for Fis1 shown by this phosphorylated protein in vitro (125). Therefore, calcium can activate DRP1 recruitment to the outer mitochondrial membrane through two mechanisms: by dephosphorylation of splice variant 1/brain isoform in serine-637, probably by calcineurin, and by phosphorylation by CaMKIα of isoform 3 in serine-600 (Fig. 10). Of note, it is surprising that phosphorylation of the same serine residue (catalyzed by distinct protein kinases) in different human DRP1 isoforms (serine-637 of splice variant 1/brain isoform and serine-600 of isoform 3) leads to opposite effects in terms of DRP1 recruitment and mitochondrial fission. As splice variant 1 is expressed only in brain, it would be of interest to study if the phosphorylation by PKA and Cdk1/Cyclin has the same effects on DRP1 isoform 3. One could hypothesize that a tissue-specific expression of DRP1 isoforms is linked to an opposite pattern of mitochondrial recruitment as a result of DRP1 phosphorylation.

Both cAMP and calcium are key second messengers in the control of cellular metabolism. Consequently, the studies presented above have provided the first mechanistic evidence that intracellular signals mediating metabolic changes rapidly alter mitochondrial morphology, or at least DRP1 activity. In addition, calcineurin and CaMKI could partly explain the DRP1-catalyzed mitochondrial scission induced by apoptotic calcium release from the ER (38).

B. FIS1

Mammalian Fis1 was also identified by homology with its yeast ortholog Fis1p (148, 285, 325). FIS1 is detected mainly on mitochondria and is inserted on the outer membrane (Fig. 5*A*). Its pattern of localization, as shown by immunofluorescence, clearly differs from that of DRP1, as FIS1 signal is dispersed throughout the mitochondrial network (148, 285, 325). The overexpression of FIS1 causes mitochondrial fragmentation, and its repression by siRNA leads to mitochondrial elongation, thereby also confirming the role of this protein in mitochondrial fission in mammalian cells (148, 285, 325). Fis1 is ubiquitously expressed and does not show large differences in its levels of expression among the different tissues assayed (151, 285). FIS1, like DRP1, is also detected on peroxisomes and regulates the fission of this organelle (173).

1. Protein domains and activity

FIS1 is a small protein of 17 kDa and 152 amino acids (see Fig. 9), and no other isoforms have been described to date. FIS1 is less conserved than DNM1L in chordates (see Table 3 and Fig. 11), and the percentage of sequence identity between species is similar to that observed in mitochondrial fusion proteins (Table 2). The mechanism by which the yeast ortholog Fis1p is inserted on the outer mitochondrial membrane has been unraveled recently (164). Interestingly, its insertion does not depend on any of the known mitochondrial machineries of protein import. Surprisingly, it depends on the lipid composition of the outer mitochondrial membrane, as supported by the observation that the insertion is inhibited by high concentrations of ergosterol (164). The domain responsible for FIS1 localization on the outer mitochondrial membrane is the COOH-terminal part, which contains an α-helix (104-122 aa), a transmembrane domain (123-147 aa), and a COOH-terminal tail exposed to the intermembrane space (148-152 aa) (Figs. 5*A* and 9) (148, 151, 285, 325). Therefore, this domain could be sensitive to the lipid composition of the mitochondrial membrane.

The NH_2-terminal part of the protein contains four differentiated regions with five α-helices (81, 288). The first 32 amino acids of rat Fis1 contain the first α-helix, which is charged and is crucial for its mitochondrial fission activity (151). The first helix has a key role in Fis1 oligomerization (151). After this first helix, there are two tetratrico-peptide repeat peptides (TPR1 33-69 aa and TPR2 70-103 aa), which contain four α-helices. These TRP-like domains are involved in protein-protein interactions and are conserved in chordates (Figs. 9 and 11). However, these domains are not required for Fis1 oligomerization, thereby suggesting that other proteins required for fission (such as Drp1, directly or not) interact with Fis1 through

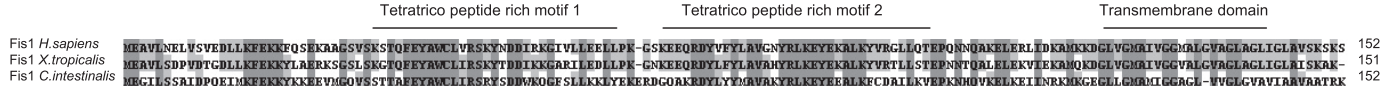


FIG. 11. Alignment of Fis1 sequences from several chordate species and the ancient chordate *Ciona intestinalis*. Alignments were performed with Clustal W on-line software. Residues are shown in intensities of gray in function of their identity through *Homo sapiens*, *Xenopus tropicalis* (frog), and *Ciona intestinalis* (prochordate). Dark gray shows the residues found in all the species analyzed, and light gray shows the residues found only in some of them. The human FIS1 protein domains are shown. Sequences used are as follows: FIS1 *H. sapiens* (NP_057152.2), Fis1 *X. tropicalis* (NP_001123678.1), and Fis1 *C. intestinalis* (XP_002121705.1).

these domains. Indeed, in the yeast ortholog of FIS1, TPR1 and TPR2 are responsible for interaction with the adaptors Mdv1 and Caf4 (331). In agreement with these data, the disruption of these domains blocks the mitochondrial fission activity of Fis1 (151).

2. Physiological role of FIS1 activity

Fis1 is thought to be a limiting factor of mitochondrial fission, as its overexpression induces mitochondrial fragmentation. In contrast, the overexpression of DRP1 does not always split the mitochondrial network, which is probably dependent on its overexpression levels and on recruitment to mitochondria (148, 182, 289). FIS1 overexpression also causes cytochrome *c* release that is dependent on DRP1 activity, thereby suggesting that FIS1-mediated mitochondrial fission also stimulates apoptosis (148, 181). Furthermore, FIS1 depletion produces an apoptosis-resistant phenotype, again confirming its role in programmed cell death (181). However, there are FIS1-induced apoptotic steps that are independent of mitochondrial fission or DRP1 activity, as the inhibition of DRP1 through the DRP1 K38A mutant does not prevent FIS1-stimulated cell death (148, 181). Some clues about this disassociation were given in the study by Alirol et al. (4), in which FIS1 overexpression fragmented mitochondria without inducing apoptosis in *Bax/Bak* double KO cells. Moreover, the capacity of FIS1 to induce apoptosis was rescued when the ER calcium levels were restored in these cells. These data suggest two independent functions of FIS1: one in apoptosis (in which the intramitochondrial COOH-terminal region of FIS1 plays a key role) and a second one in mitochondrial fission (4).

Furthermore, studies on FIS1 overexpression at short times show that mitochondrial fragmentation can occur in the absence apoptosis, and with no changes in either mitochondrial membrane potential or in calcium fluxes (4, 102). However, FIS1 overexpression also causes a higher reduction in mitochondrial membrane potential during longer incubations with oligomycin, thereby suggesting enhanced susceptibility to complex V inhibition (4). However, these studies were performed in HeLa or MEF cells and do not discard a key role of mitochondrial architecture and/or FIS1-mediated calcium fluxes in other cell types or in tissues.

The other two cellular processes regulated by FIS1 activity, which are probably related, are senescence and mitophagy (autophagy of mitochondria). Cellular senescence has been linked to an increase in mitochondrial length (180, 328). This increase was explained by a marked reduction in FIS1 expression and a slight increase in MFNs expression (180, 328). Furthermore, the specific modulation of Fis1 mediates changes in the senescent phenotype (180, 328). Therefore, the overexpression of FIS1 inhibits senescence, whereas overexpression of inactive DRP1 (K38A) and an inactive form of FIS1 enhances this process (180, 328). Moreover, specific repression of FIS1 causes a marked phenotype of senescence, with a decreased rate of proliferation together with cellular shape changes and granularity (328). Importantly, the observation that OPA1 repression reverts the senescent phenotype caused by FIS1 depletion demonstrates that ongoing mitochondrial fusion is crucial to induce senescence (180). Impaired mitochondrial function may be the factor responsible for triggering senescence. This notion is supported by the observation of a decrease in the mitochondrial membrane potential and an increase in ROS production in FIS1-depleted cells (180).

The mechanism by which defective mitochondrial activity causes cellular senescence may be explained by a functional interaction between mitochondrial dynamics and autophagy. In this regard, it has been reported that a functional mitochondrial fission machinery is required to discard damaged mitochondria from the network, which are then removed by autophagy (112, 302). Thus overexpression of FIS1 triggers mitophagy and depletion of Fis1 or overexpression of DRP1 K38A attenuates this process (112, 302). This reduction in mitophagy causes the continuous presence of damaged mitochondria, which disturbs the function of the entire mitochondrial network (302). Thus, under these conditions, there is an increase in oxidized mitochondrial proteins and less maximal activity of the respiratory chain in Fis1-depleted or DRP1K38A cells (302), which has additional functional consequences in beta-pancreatic cells.

IV. OTHER PROTEINS INVOLVED IN MITOCHONDRIAL DYNAMICS

The following proteins that we review have been demonstrated to control mitochondrial fusion and fission; however, the mechanisms are unclear. In addition to the canonical mitochondrial fusion and fission proteins (MFN1, MFN2, OPA1, FIS1, and DRP1), all of the proteins described below show properties of interest that may contribute to elucidating the role and regulation of mitochondrial dynamics in mammals.

GDAP1 (ganglioside-induced differentiation activated protein 1) is a mitochondrial protein, anchored on the outer mitochondrial membrane, which is expressed mainly in nerve tissues (192, 218, 232). Depletion of GDAP1 causes an elongation of mitochondrial tubules. In contrast, overexpression of GDAP1 leads to mitochondrial fragmentation, which can be reversed by overexpression of Drp1 K38A and gain-of-function of both Mfns (218, 232). Importantly, GDAP1-induced mitochondrial fission does not alter the rate of mitochondrial fusion, as demonstrated by the equal exchange of mitochondrial matrix content in GDAP1-overexpressing cells (218).

This protein contains two separate glutathione-*S*-transferase (GST) domains before a COOH-terminal bipartite hydrophobic domain, which bears the mitochondrial-targeting signal (218). Although possessing GST domains, GDAP1 does not show glutathione transferase activity (232). The mechanism by which GDAP1 controls mitochondrial morphology is not known.

MTP18 (mitochondrial protein 18 kDa) has been described as a target of phosphatidylinositol (PI) 3-kinase as MTP18 expression depends on activated signaling of this kinase. MTP18 shows high levels of expression in heart and skeletal muscle but is also detected in other tissues. Of note, this protein has no ortholog described in yeast. MTP18 is located inside the mitochondria; more precisely, it is anchored to the outer or the inner membrane facing the intermembrane space. Overexpression and repression studies demonstrate that MTP18 activates mitochondrial fission (298, 299). Like GDAP1, inhibition of DRP1 activity and increase in MFN1 activity compensate the mitochondrial fission caused by MTP18. Interestingly, data support the notion that MTP18 controls DRP1 distribution on mitochondria and that FIS1 fission activity is also modulated by MTP18. Although MTP18 repression increases mitochondrial length, it also induces cytochrome *c* release and apoptosis, and thus represents one of the first descriptions that mitochondrial elongation also enhances cytochrome *c* release and apoptosis (298, 299). In all, MTP18 may be one of the strongest key candidates of the unknown inner membrane fission machinery.

Endophilin B1/Bif 1 was considered a fatty acyl-transferase that controls mitochondrial morphology (158). However, several lines of evidence demonstrate that endophilin B1 does not show acyl-transferase activity in endocytosis or Golgi fission, and its major activity is the deformation of membranes through its BAR domain (104, 105). Regarding its role controlling mitochondrial morphology, loss of 80% of endophilin B1 selectively affects and inhibits outer membrane but not inner membrane fission (159). Moreover, cells with downregulation of both Drp1 and endophilin B1 show the same alteration in mitochondrial morphology as Drp1 downregulated cells, thereby suggesting that Drp1 acts upstream of endophilin B1 (158). In contrast, complete loss of endophilin B1/Bif1 (Bif1 −/− mouse) reduces Bax/Bak activation and cytochrome *c* release upon induction of apoptosis, without affecting mitochondrial morphology (291). Endophilin B1/Bif1 is an interesting protein as it may be involved in modifications that change the lipid composition and/or curvature of the outer mitochondrial membrane, and thus it may contribute to an additional mechanism of control of mitochondrial fission. However, further work is required to explain the discrepancies in the regulation of mitochondrial dynamics by endophilin B1.

The phospholipase activity of mitochondrial phospholipase D (mitoPLD) is required for normal mitochondrial fusion mediated through Mfns. This phospholipase hydrolyzes cardiolipin to generate phosphatidic acid. However, it remains to be determined how this hydrolytic activity and its products regulate mitochondrial fusion (55).

The outer mitochondrial membrane protein MULAN (mitochondrial ubiquitin ligase activator of NFκB) modulates mitochondrial morphology possibly by increasing mitochondrial fragmentation. However, the clearest role exerted by MULAN is the regulation of mitochondrial distribution. Interestingly, the catalytic domain of this ubiquitin ligase is exposed towards the cytosol, and MULAN was initially described as an activator of the transcription factor NFκB, which has a key role in immune response and inflammation (187). Although there are several reports that relate NFκB signaling with mitochondria (203, 264), MULAN is the first protein that directly links NFκB and regulation of mitochondrial morphology and distribution. In addition, this novel mitochondrial protein is postulated to mediate mitochondria-to-nucleus signaling. The mechanisms by which MULAN controls mitochondrial morphology and increases mitochondrial fragmentation have not been described.

The protein Miro is an atypical Rho GTPase that contains tandem GTP binding domains linked by a region with putative binding sites for calcium (EF-hand motifs). Miro1 was identified in yeast, named Gem1p, and described to control mitochondrial morphology (100). Its main activity identified to date is the control of mitochondrial trafficking and distribution (120), although several evidences show that it is also involved in mitochondrial fusion and fission (98, 257).

The protein Mff (mitochondrial fission factor) mediates mitochondrial and peroxisome fission in a similar manner as Fis1 and Drp1. However, Mff is located in the outer mitochondrial membrane and not in the same complexes as Fis1, thereby suggesting an independent role of Mff in fission (106).

V. MITOCHONDRIAL DYNAMICS AND MITOCHONDRIAL FUNCTION

In the following sections we analyze the evidence currently available that links the operation of mitochondrial dynamics proteins and mitochondrial function. Some aspects related to mitochondrial function have been addressed in previous sections, and here we focus on two basic aspects, namely, mitochondrial metabolism and the maintenance of mitochondrial DNA.

A. Role of Mitochondrial Dynamics in Mitochondrial Metabolism

Studies performed before the identification of the proteins involved in mitochondrial fusion or fission re-

ported that mitochondrial filaments conduct mitochondrial membrane potential in fibroblasts and in cardiomyocytes (7, 272). On the basis of these observations, it was proposed that the electrical activity of the mitochondrial network in large cells, such as muscle fibers, permits the movement of energy from the cell periphery to the cell core. If this were true, mitochondrial function would depend on the level of activity of mitochondrial dynamics. More recent data demonstrate that mitochondrial matrix continuity in the whole mitochondrial network is not total, and some mitochondria may be only tethered or connected, but not completely fused (303). Therefore, distinct levels of regulation (in addition to fusion and fission) of the mitochondrial network are likely to occur.

During recent years, information has been gathered on the impact of alterations in the activity of certain mitochondrial dynamics proteins on mitochondrial energization and mitochondrial function. Substantial evidence has been obtained in mammalian cells after the manipulation of Mfn2 expression. Thus stably transfected fibroblasts with a Mfn2 antisense sequence show reduced glucose oxidation and a similar reduction in oxygen consumption (16). In these conditions, coupled oxygen consumption is unaltered, whereas respiration linked to the mitochondrial proton leak is significantly reduced in antisense cells (16). The use of a distinct cell model also provided a similar metabolic profile, and Mfn2 repression mediated by adenoviral antisense expression in L6E9 myotubes also reduces glucose oxidation (16, 236). The effect detected in L6E9 muscle cells is specific, as infection with an adenoviral vector encoding β-galactosidase does not alter glucose oxidation. Under these conditions, glucose transport and lactate production are markedly enhanced in antisense cells, whereas glucose incorporation into glycogen is significantly depressed (236). Mfn2 repression is also associated with decreased rates of pyruvate or palmitate oxidation in L6E9 muscle cells (236) and causes a reduction in mitochondrial membrane potential in the presence of several oxidative substrates (16, 236). The alterations in substrate oxidation induced by Mfn2 loss-of-function are not explained by a lower mitochondrial mass, and *N*-acridine orange labeling or the abundance of the mitochondrial protein porin remain unaltered under these conditions.

These data indicate that Mfn2 knockdown causes alterations in mitochondrial metabolism characterized by reduced mitochondrial membrane potential and cellular oxygen consumption as well as depressed substrate oxidation (Table 4). Mfn2 loss-of-function reduces the activity of Krebs cycle and of the electron transport chain in such a way that the energy metabolism of the cell is compensated by a higher rate of glucose uptake and glycolysis and a lower rate of glycogen synthesis. The cell with low Mfn2 activity relies on the use of anaerobic glycolysis to generate energy. These results are in keeping with the observations made in MEFs with targeted null mutations of both *Mfn1* and *Mfn2*. These cells show loss of mitochondrial membrane potential, reduced endogenous respiration, and an incapacity to increase respiration upon the addition of the ionophore 2,4-dinitrophenol (50). Respiration is normalized by overexpression of Mfn2 (50).

TABLE 4. *Role of mitochondrial dynamics in cell metabolism and proliferation*

Metabolic Effects	Reference Nos.
Inhibition of mitochondrial fusion (Mfn2/OPA1) or inhibition of fission (Drp1)	16, 25, 50, 223, 236
Low mitochondrial $\Delta\psi$	
Reduction of oxygen consumption	
Low activity of respiratory complexes	
Effects of Mfn2 repression in muscle cells	16, 236
Reduction in glucose and palmitate oxidation	
Low abundance of OXPHOS subunits (I, II, III, and V)	
Effects of Mfn2 gain-of-function independent of fusion activity (Mfn2 Δ602-757)	236
High glucose oxidation	
High mitochondrial $\Delta\psi$	
Cell proliferation	
Increase in Mfn2 or Fis1 inhibition (increase in mitochondrial length)	53, 180
Reduction in cell proliferation	

Cells with normal mitochondrial dynamics show normal rates of respiration and glucose oxidation and a defined activity of mitochondrial respiratory complexes. Mfn2 loss-of-function causes reduced mitochondrial respiration and oxidation of glucose, decreased mitochondrial membrane potential, and diminished oxidative phosphorylation because of reduced expression of subunits that comprise the respiratory complexes. OPA1 loss-of-function, which blocks mitochondrial fusion, also causes reduced mitochondrial respiration, decreased mitochondrial membrane potential, and diminished activity of mitochondrial respiratory complexes. Overexpression of a dominant negative mutant of Drp1 (Drp1 dn) that blocks mitochondrial fission also causes reduced oxygen consumption and low activity of respiratory complex IV. Overexpression of a truncated form of Mfn2 (Mfn2Δ602-757) enhances mitochondrial oxidation of glucose and increases mitochondrial membrane potential and OXPHOS subunit expression. Mfn2 overexpression or Fis1 loss-of-function, manipulations that either enhance mitochondrial fusion or block mitochondrial fission, also lead to reduced cell proliferation.

Human fibroblasts from patients showing certain MFN2 mutations also show metabolic alterations. Thus fibroblasts from R364Q or A166T mutants show enhanced basal oxygen consumption, normal coupled respiration and enhanced oligomycin-insensitive respiration, and reduced mitochondrial membrane potential (193). Permeabilized mutant cells show normal rates of ATP synthesis and low coupling efficiency (ATP/O ratio) (193). These changes occur under conditions in which mitochondrial mass is unaltered and the activity of oxidative phosphorylation (OXPHOS) complexes is normal in mutant fibroblasts (193). Other MFN2 mutants, such as M21V, T105M, I213T, or V273G, do not show metabolic alterations (8, 193). In all, some MFN2 mutations cause metabolic alterations in human fibroblasts via mechanisms that are currently unknown.

Alterations in OPA1 expression also affect mitochondrial metabolism. OPA1 knockdown induced by RNAi in HeLa cells causes a marked reduction in mitochondrial membrane potential (223) and, similarly, depletion of OPA1 by RNAi in MEFs leads to widespread loss of this potential (50). In addition, OPA1 depletion in MEFs causes a reduction in basal respiration and incapacity to enhance oxygen consumption in the presence of the uncoupler 2,4-dinitrophenol. Respiration is normalized by overexpression of OPA1 (50). Human fibroblasts from patients with certain OPA1 mutations (that cause autosomal dominant optic atrophy or ADOA) (c.2708delTTAG, c.1705 + 1G4T, c.1516 + 1G4, c.2819-2A4C, c.1346_1347insC) show impaired ATP synthesis driven by complex I substrates and decreased rates of mitochondrial fusion (330). In contrast, other studies performed with cells from ADOA patients with distinct mutations (c1410_144314del38, c.239A>G, c.2883A>C, c.2522A>G, c.2780T>A, c.1654delT, c.1929delC, c.2708delTTAG) show normal mitochondrial activity and bioenergetics (202, 280). The basis for this discrepancy remains unknown, although it is unlikely that this is due to alterations specific to the different mutations.

The data we have summarized so far indicate that deficiency in protein components of mitochondrial fusion (mainly MFN2 and OPA1) and/or in mitochondrial fusion per se reduces mitochondrial membrane potential and respiration in several cell types (Table 4). Current evidence indicates that alterations of mitochondrial fission proteins produce similar effects on mitochondrial metabolism. Thus RNAi of DRP1 in HeLa cells causes a reduced basal rate of oxygen consumption (in the presence of galactose in the medium), reduced coupled respiration, and a lower rate of ATP synthesis (in permeabilized cells) (25). Under these conditions, repression of DRP1 is associated with lower rates of state 4 and state 3 respiration and reduced complex IV activity (25). These deleterious effects on mitochondrial metabolism have been reproduced in a second study, in which DRP1 ablation in mammalian cells also has a negative effect on mtDNA nucleoid homeostasis (discussed in the next section) (228). Expression of a dominant negative mutant form of DRP1 also causes a marked reduction in the respiratory capacity of INS1 rat insulinoma cells (302). RNAi-mediated Fis1 depletion also reduces the maximal respiratory activity of INS1 cells, and overexpression of FIS1 rescues the phenotype (302). Hence, it can be concluded that perturbation of mitochondrial network dynamics, via fusion or fission disruption, induces the impairment of mitochondrial energy production in mammalian cells (Table 4).

Relatively little is known about the mechanisms by which disruption of mitochondrial fusion or fission alters mitochondrial energy production. Most studies have focused on the expression or activity of respiratory complexes. Mfn2 knockdown in L6E9 muscle cells causes a marked repression of the expression of the subunit p39 from complex I (encoded by nuclear DNA), protein p70 from complex II (encoded by nuclear DNA), p49 (core 2 subunit encoded by nuclear genome) from complex III, and the α-subunit of complex V (encoded by nuclear DNA) (236). Under these conditions, no alterations in the abundance of Cox1 (encoded by nuclear DNA), Cox4 subunits of complex IV (encoded by mitochondrial DNA), or porin (encoded by nuclear DNA) are detected (236). In parallel, the enzymatic activity of respiratory complexes I+III or III decrease in Mfn2 knockdown cells (236). Deficient activity of complexes I, II, and III is also detected in permeabilized Mfn double KO cells (50). These data indicate that Mfn2 loss-of-function causes a specific alteration in the expression of subunits that participate in complexes I, II, III, and V, which leads to reduced activity of several components of the OXPHOS system (Fig. 13). These changes are not a consequence of alterations in the expression of the nuclear coactivator Ppargc1a (PGC-1α). Deficient activity of complexes I, II, and III is also detected in permeabilized Opa1-deficient MEFs (50), but there is no information on the mechanisms involved.

A relevant question from the point of view of the mechanisms involved in the regulation of mitochondrial metabolism by mitochondrial dynamics proteins is whether gain-of-function also exerts biological effects. This question has been analyzed to some extent for MFN2. Overexpression of MFN2 in HeLa cells causes perinuclear aggregation of mitochondria, a marked enhancement of mitochondrial membrane potential and increased glucose oxidation (236). MFN2 gain-of-function in L6E9 myoblasts is also associated with increased expression of several subunits of complexes I, IV, and V (236). In contrast to the effects of MFN2, overexpression of Opa1 in MEFs does not modify mitochondrial metabolism (50).

To determine whether the effects induced by MFN2 gain-of-function on mitochondrial activity are related to mitochondrial fusion activity, studies have been performed with a COOH-terminal truncated form of MFN2 (hMfn2Δ602-757), which no longer has the capacity to induce mitochondrial fusion. Overexpression of hMfn2Δ602-757 in HeLa cells does not alter the morphology of mitochondrial filaments, and it is localized mainly in mitochondria but is also present in the cytosol (20% of total). Truncated MFN2-overexpressing cells show a marked enhancement of mitochondrial membrane potential and stimulation of glucose oxidation (236). In keeping with these observations, inspection of mitochondria under an electron microscope indicates that the expression of the truncated MFN2 version induces morphology consistent with a condensed state. In all, the evidence available indicates that MFN2 gain-of-function activates mitochondrial metabolism and increases the expression of subunits of the OXPHOS system. These stimulatory effects of MFN2 on

mitochondrial metabolism are not secondary to its effects as a mitochondrial fusion protein.

On the basis of the information currently available, we propose that mitochondrial network dynamics proteins affect mitochondrial metabolism through two distinct pathways: *1*) perturbation of mitochondrial network dynamics, via fusion or fission disruption, induces the impairment of mitochondrial energy production in mammalian cells, which suggests that mitochondrial dynamics by itself controls mitochondrial metabolism (Table 4); and *2*) MFN2 seems to play a specific regulatory role in mitochondrial metabolism through mechanisms that involve signaling that causes changes in the expression of OXPHOS subunits (Table 4). Specific analysis of the molecular mechanisms involved in the two pathways is required. In addition, demonstration of how these pathways operate in in vivo conditions is mandatory. A very recent study, however, allows us to put forward a hypothesis about the specific metabolic effects of Mfn2 and the specific signals originated as a result of its specific loss-of-function and not only by changes in mitochondrial fusion. This report demonstrates that Mfn2, and not Mfn1, tethers mitochondria to the ER and this interaction is crucial for intracellular calcium fluxes (72). Calcium is a key mediator of intracellular signaling and also a known activator of the Krebs cycle enzymes, or transcription factors through activation of kinases, phosphatases, or other mechanisms. Therefore, alterations in calcium fluxes caused by the lack of Mfn2 and the alteration of mitochondria-ER contacts can lead to Mfn2-specific metabolic alterations signaled by calcium.

B. Mitochondrial Dynamics and Mitochondrial DNA

In mammals, mitochondrial DNA (mtDNA) is a 16.5-kb circular double-stranded DNA (10, 30) present in one to several thousand copies per cell (292). MtDNA in the yeast *S. cerevisiae* appears to be organized in discrete foci, called nucleoids, within mitochondria (212). Although mtDNA inheritance differs significantly between budding yeast and mammalian cells (obligate aerobic), there is genetic and cell biology evidence to believe that mtDNA is also organized in nucleoid-like structures in mammals (146, 185). Studies on living cells stained with bromodeoxyuridine (BrdU) have revealed a punctate intramitochondrial distribution of mtDNA (183), as well as the capacity of mtDNA to diffuse into the mitochondria of $\rho 0$ cells (127). MtDNA is also detected in punctate structures on fixed cells (107, 195, 200). It has recently been reported that mtDNA of human primary and immortal cells is organized in hundreds of nucleoids (500–800 nucleoids/cell) that are enriched in mitochondrial transcription factor A (TFAM) (183). Nucleoids contain a mean of 2–8 mtDNA molecules and are distributed throughout the entire mitochondrial compartment (183). However, nucleoids show a lower motility in the mitochondrial compartment compared with other mitochondrial matrix proteins (183). This difference in motility suggests a specific regulation of nucleoid distribution and/or a specific restriction in nucleoid dynamics (183).

The specific colocalization of Twinkle with mtDNA in punctate intramitochondrial structures demonstrates the presence of discrete mitochondrial DNA-protein complexes in human mitochondria, similarly to yeast (279). The colocalization of mtDNA with endogenous TFAM as well as with tagged forms of Twinkle (107), a putative helicase, suggests that DNA-positive structures are nucleoprotein complexes homologous to the mtDNA nucleoids of yeast. An initial characterization of the composition of human mitochondrial nucleoids revealed that TFAM, mitochondrial single-stranded DNA-binding protein (mtSSB), and Twinkle colocalize in intramitochondrial nucleoids (detected by the specific incorporation of BrdU) (107). Furthermore, mtDNA polymerase POLG (mtDNA polymerase γ) copurifies with mtDNA nucleoids (107).

In addition to their specific catalytic roles, the proteins associated with mtDNA in nucleoids participate in the maintenance of mtDNA. This is supported by observations that mutations in genes encoding some of the proteins found in nucleoids result in large mtDNA rearrangements and an abnormal copy number of the mitochondrial genome. In this regard, mutations in genes encoding Twinkle (*PEO1*) (174, 279), the catalytic and accessory subunits of mtDNA polymerase γ (*POLG1* and *POLG2*) (194, 305), cause the autosomal dominant progressive external ophthalmoplegia (adPEO), an adult-onset mitochondrial disorder characterized by ophthalmoparesis with exercise intolerance, ataxia, peripheral neuropathy, and multiple mtDNA deletions. In addition, mutations in genes *PEO1* (Twinkle) (258) or *POLG1* (94) have been detected in the so-called mtDNA depletion syndrome (MDS), a clinically and genetically heterogeneous group of autosomal recessive diseases characterized by a reduction in mtDNA copy number.

The link between mitochondrial dynamics and mtDNA maintenance originally came from studies in yeast. Thus wild-type function of Fzo1 (an ortholog of mammalian Mfns) is required for the maintenance of mtDNA in *S. cerevisiae* (130, 247). Similarly, deletion of Mgm1 (ortholog of mammalian OPA1) leads to loss of mtDNA in yeast (117, 154, 267). In keeping with these observations, MEFs lacking Opa1 show that the majority of mitochondria lacked mtDNA nucleoids (52). Perhaps also in connection with these findings, it has been reported that some OPA1 missense mutations cause the accumulation of multiple mtDNA deletions in human skeletal muscle (6, 140). In contrast, MEFs lacking Mfn2 or Mfn1 contain a significant fraction of mitochondria with-

out nucleoids but without significant loss of mtDNA as measured by Southern blot (51, 52). In all, current evidence indicates that normal activity of OPA1 or MFNs is crucial to maintain mtDNA nucleoids. The mechanisms involved are unknown, and a major hypothesis to be tested is whether OPA1/MFN complexes bind to mitochondrial nucleoids. The topology of OPA1 is not entirely consistent with a potential direct binding to mitochondrial nucleoids, as OPA1 isoforms anchored in the inner membrane expose the major part of the protein to the intermembrane space. However, OPA1, through its putative transmembrane domain, could participate in the same complexes as mtDNA nucleoids through additional partners. Moreover, direct binding with nucleoids could be also hypothesized as anchored OPA1 isoforms contain some residues before the putative transmembrane domain that could be exposed to the matrix (10 residues from 87-97, Fig. 4). There was no evidence supporting a role of mitochondrial fission proteins in mtDNA stability. Indeed, DRP1 does not colocalize with nucleoids (107). However, a study published during the revision of this manuscript reports that DRP1 loss-of-function in mammalian cells causes a loss of mtDNA (228). Although the mechanism of this loss remains unknown, this study demonstrates that alterations in mtDNA nucleoid homeostasis caused by changes in the components of mitochondrial dynamics are more likely due to alterations in the proper balance of mitochondrial fusion and fission events, and not only to selective alterations in mitochondrial fusion.

VI. ROLE OF MITOCHONDRIAL DYNAMICS ON CELL PROLIFERATION AND APOPTOSIS

A. Mitochondrial Dynamics and Cell Proliferation

Several lines of evidence support the view that MFN2 exerts a regulatory role on the cell cycle. Mfn2 (named in the original study as "hyperplasia suppressor gene", HSG) is repressed in VSMCs under conditions of hyperproliferative states such as atherosclerosis or restenosis (53). Moreover, Mfn2 overexpression in VSMCs causes growth arrest characterized by an increased number of cells in G_0/G_1 stage and a reduction of cells in S or G_2/M phases (53). Overexpression of Mfn2 also inhibits VSMC proliferation induced by exposure to oxidized low-density lipoproteins (121). Under in vivo conditions, adenovirus-mediated Mfn2 overexpression prevents restenosis associated with balloon angioplasty in rats (53) and reduces atherosclerotic lesion formation in rabbits treated with a high-cholesterol diet (121). These studies have potentially relevant clinical implications, as the proliferation of VSMCs is critical in atherosclerotic heart disease and during coronary artery restenosis secondary to balloon angioplasty. There is also preliminary evidence that supports a potential role of Mfn2 in mouse blastocyst development (150).

The mechanism proposed by which Mfn2 causes cell cycle arrest involves binding and sequestration of Ras, thereby inhibiting the downstream Raf-MEK1/2-ERK1/2 signaling pathway (Fig. 12). In this regard, deletion of a $p21^{ras}$ signature motif located at aa 77-92 (N-DVKGYLSKVRGISEVL-C) abolishes the inhibitory effects of Mfn2 on ERK1/2 and cell growth (53). This analysis is consistent with the observation that dominant-negative Ras expression inhibits VSMC proliferation in vivo (321) and that approaches that inhibit the entry of VSMCs into the cell cycle prevent restenosis secondary to the implantation of mechanical stents in patients with chronic coronary artery disease (86, 211).

In keeping with an antiproliferative role of MFN2, FIS1 depletion in Chang cells reduces cell proliferation in parallel with an enhancement in the mitochondrial network (180). Additional support for a link between cell proliferation and mitochondrial dynamics comes from the observation that the deficient cell growth detected in Phb2-deficient cells is partially restored upon expression of a noncleavable OPA1 variant (206). Although it is still early to draw definitive conclusions, current data suggest a potential role of mitochondrial dynamics in cell cycle (Table 4), in which Mfn2 shows an antiproliferative action. Whether the effects of MFN2 are universal in different cell types and species is unknown. Further studies are required to address whether MFN2 participates in disorders other than in VSMCs and whether it is linked to tumor growth.

B. Mitochondrial Dynamics and Apoptosis

The effects of apoptosis on mitochondrial morphology were established before the identification of the genes involved in mitochondrial dynamics (reviewed in Ref. 201). However, it was not until the 1990s that the relevance of mitochondria as key organelles controlling apoptosis was established. Indeed, one of the two major path-

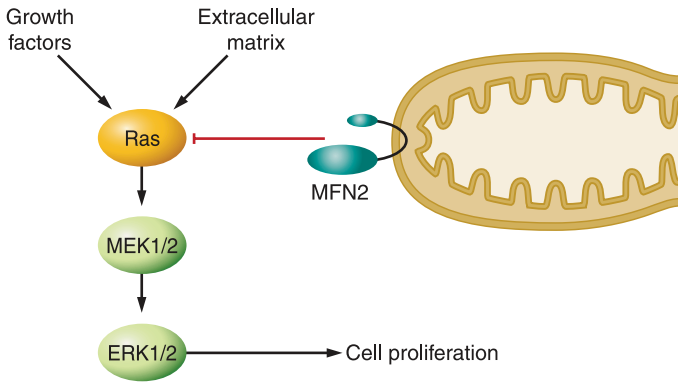


FIG. 12. Mechanism proposed by which Mfn2 inhibits cell proliferation. Mfn2 overexpression inhibits Ras and the cell cycle at the G_0/G_1 transition in vascular smooth muscle cells.

ways of apoptosis activation, the intrinsic pathway, is also known as the mitochondrial pathway. The step of this pathway, considered as "the point of no return," or when reached irreversibly causes cell death, is the permeabilization of the outer mitochondrial membrane (176).

One of the main accepted mechanisms of mitochondrial membrane permeabilization is the formation of pores by BAX and BAK proteins. BAX is located in the cytosol and BAK in the outer mitochondrial membrane. When BAX and BAK are activated by apoptotic stimulus, the former translocates from the cytosol to the mitochondria and forms a pore in the outer membrane, alone or together with BAK, which allows the release of proapoptotic proteins. Furthermore, BAX and BAK are inhibited by the BCL2 antiapoptotic proteins (such as BCL2 itself and BCLX), which are also located in mitochondria (176).

Importantly, not only does the formation of distinct types of pores regulate this release, but the morphology of mitochondrial cristae also plays a relevant role, as it controls the compartmentalization of some of these mitochondrial cell-death effectors and, therefore, their release to the cytosol (197, 198, 260). Electron tomography has allowed the three-dimensional study of mitochondrial cristae, the determination of cristae junctions (key structures in this compartmentalization of cell-death effectors) and their changes during distinct cellular processes (101, 197, 198). Thus the dynamics and remodeling of mitochondrial cristae is a potential key point of control of intrinsic apoptosis in which mitochondrial dynamic components could play a relevant role. This relevant issue of mitochondrial cristae organization and remodeling in several situations has been recently reviewed in depth (334).

Permeabilization of mitochondria causes cell death by several mechanisms. The first is the release of proteins from the mitochondria that are functionally cell-death effectors in the cytosol. These effectors can be grouped into two types: those that induce programmed cell death through the activation of caspases (the proteases that execute the cell destruction characteristic of the programmed cell death), such as cytochrome *c*, and those that induce this process independently of caspase activity, such as apoptosis-inducing factor (AIF). A second major mechanism is the loss of the mitochondrial membrane potential as a result of membrane permeabilization, a failure of the OXPHOS, in which the cytochrome *c* plays an active role, and a concomitant reduction in ATP synthesis, together leading to a bioenergetic catastrophe that contributes to cell death (176).

1. Regulation of apoptosis by mitochondrial fusion and fission components

In this context, the first evidence that mitochondrial dynamics modulates programmed cell death was provided by Frank et al. (97). First of all, this study demonstrated that apoptosis causes mitochondrial fragmentation (97). Given that apoptosis was previously shown to induce mitochondrial permeabilization and bioenergetic failure, one could expect that this would lead to a decrease in mitochondrial fusion, as a result of a dissipation of the mitochondrial membrane potential and the consequent bioenergetic failure. Or, what is the same, that mitochondrial fragmentation is a consequence of apoptosis. However, key lines of evidence demonstrated that this fragmentation during apoptosis is a tightly regulated process. The first was that mitochondrial fragmentation occurs before the activation of the caspases. Thus it is not a late apoptotic event or a consequence of caspase activity. The second, and most important, line of evidence was that overexpression of a dominant negative mutant form of DRP1 that elongated mitochondria inhibits cytochrome *c* release and delays the apoptotic response (97). Therefore, alteration of mitochondrial dynamics through DRP1 delays and even arrests (147) apoptotic progression in some nonmammalian models. Taken together, these data demonstrated that mitochondrial fission is an upstream and conserved event that participates in apoptosis, although it remained to be established whether it was the cause of apoptosis or concomitant to apoptosis progression (by enhancing or delaying its progression).

Later on, it was also demonstrated that a decrease in mitochondrial fusion as a result of OPA1 depletion, or an increase in mitochondrial fission by overexpression of FIS1, is also sufficient to enhance apoptotic progression (12, 116, 148, 181, 223). Thus this finding pointed to mitochondrial morphology or fusion and fission rates being the key parameters to enhance apoptosis. Furthermore, Mfn2 has been shown to interact with Bak and with antiapoptotic BCL2 and BCLX (39, 77). Indeed, Bak and Bax also control mitochondrial morphology through Mfn2, where Bax inhibits Mfn2 fusion activity during apoptosis (157, 159, 160). Moreover, antiapoptotic BCLX induces mitochondrial fusion (77), which has been shown to protect against apoptosis in some models (97, 181). Also, there is a study reporting that an activated form of MFN2 that increases mitochondrial fusion reduces BAX activation (216). Therefore, key proteins that regulate outer membrane permeabilization and apoptosis also functionally interact and modulate mitochondrial fusion/fission proteins, and vice versa. Taken together, these data demonstrate that increasing the rate of mitochondrial fission or decreasing the rate of mitochondrial fusion stimulates apoptosis. Furthermore, these data indicate that modulators of apoptosis have the capacity to interact and regulate the components of mitochondrial dynamics. Thus mitochondrial fragmentation is regulated by apoptotic modulators through the inhibition of mitochondrial fusion or activation of mitochondrial fission, respectively, which could cause or enhance the progression of apoptosis.

However, an increased rate of mitochondrial fission does not always correlate with activation of apoptosis or

the opposite situation (more fusion, less apoptosis). For instance, *Mfn1* −/− and *Mfn2* −/− cells show extensive mitochondrial fragmentation and yet are fully viable (50, 51), and indeed, apoptosis is not enhanced in these cells (103). Furthermore, dissipation of the mitochondria membrane potential with the uncoupler CCCP or OPA1 overexpression result in fully viable cells showing massive mitochondrial fragmentation (182). Thus these lines of evidence demonstrate that an increase in mitochondrial fission or mitochondrial fragmentation is not sufficient to start the cell death program. These findings could be expected as mitochondrial fission occurs normally under physiological, nonapoptotic conditions. Therefore, proteins involved in mitochondrial dynamics may directly participate in apoptosis independently of their regulatory activity over mitochondrial shape.

In this regard, mitochondrial dynamics genes that stimulate apoptosis under some conditions alter mitochondrial morphology without causing changes in programmed cell death progression. FIS1 has been shown to stimulate fragmentation without inducing apoptosis (4), and DRP1-induced mitochondrial fragmentation inhibits apoptosis by blocking the transmission of calcium waves through the mitochondrial network, a triggering signal of cell death (289). Moreover, inhibition of the mitochondrial fission machinery (through FIS1 and DRP1 modulation) does not block BAX/BAK-dependent apoptosis or mitochondrial permeabilization, and only inhibits the release of cytochrome *c* but not of other apoptogenic factors (13, 229). These observations therefore suggest a contribution of fission machinery only in a specific step of apoptosis (13, 229). In this regard, repression of MTP18 elongates mitochondria, also by inhibiting fission, and stimulates apoptosis (298, 299). In conclusion, these and other reports demonstrate that mitochondrial dynamics proteins also control mitochondrial dynamics independently of its activity causing or enhancing apoptosis. Thus, again, mitochondrial fission caused by mitochondrial dynamics genes is not always linked to apoptosis.

Several mechanisms have been described that help to discern the role of DRP1 and OPA1 in apoptosis, which, in this case, are independent of their activity controlling mitochondrial fission and fusion, respectively. Basically, the main concept is that both DRP1 and OPA1 regulate mitochondrial cristae morphology, which, as mentioned, also controls the release of apoptogenic factors (101, 109, 197, 198, 260). In the case of OPA1, it directly regulates the size of cristae junctions, and this size is directly related to the capacity to release cytochrome *c* and, therefore, to activate apoptosis (101). In contrast, DRP1 activity is required for BIK-induced cristae remodeling, which causes opening of the cristae, as measured as an increase in the intracristae cross-sectional distance (109). Furthermore, the regulation of cristae remodeling by DRP1 or OPA1 is independent of the permeabilization of the outer mitochondrial membrane (101, 109).

In addition, Drp1 recruitment and/or stabilization on the outer mitochondrial membrane induced by apoptosis is regulated by several mechanisms (see sect. III*A*), some of which may be activated and enhance Drp1-mediated mitochondrial fission in nonapoptotic situations (such as an increase in PKA activity caused by cAMP, which can occur in a nonapoptotic context, catabolic conditions, etc.) (48, 68, 329).

Importantly, DRP1, MFN2, and OPA1 modulate mitochondrial metabolism and bioenergetics (16, 25, 50, 236). Thus we cannot discard a contribution to apoptosis by the bioenergetic parameters specifically regulated by mitochondrial dynamics components, which may also be independent of their role in mitochondrial morphology (236).

Taken together, all these data suggest that regulation (cause or enhancement) of apoptosis by mitochondrial dynamics relies more on the specific activity of the proteins involved in mitochondrial dynamics than on the changes in mitochondrial morphology per se. Given the tissue-specific pattern of mitochondrial activity, of mitochondrial dynamics components, and of the efficiency or abundance of some apoptotic pathways and factors, we consider that the contribution of mitochondrial dynamics to apoptosis differs greatly depending on the tissue and cellular context or stimulus, as distinct genes and opposed mechanisms are involved. Thus the tissue-specific activity of mitochondrial dynamics proteins and their mechanisms of regulation will help to reveal their contribution to apoptosis. This hypothesis is confirmed by data showing that inhibition of *C. elegans* DRP1 arrests developmental apoptosis only in a concrete subset of cells, thereby indicating that DRP1-mediated apoptosis is relevant only in a specific cellular context or tissue (147).

Finally, the exact position of mitochondrial dynamics proteins or mitochondrial fragmentation in the cascade of events of apoptosis also remains to be determined (201). Thus what is clear is that mitochondrial fragmentation is an early apoptotic event and is not a consequence of apoptosis in most of the cases reported. The observation that inhibition of mitochondrial fission in mammalian cells only delays the apoptotic progression and does not stop the release of some proapoptotic factors suggests that mitochondrial dynamics are concomitant to apoptosis and that its regulation may enhance or delay apoptosis. These lines of evidence also demonstrate that mitochondrial fission is not sufficient or essential for programmed cell death activation. Recent data also support this view. Thus the role of caspase-dependent cleavage products of mitochondrial dynamics proteins, such as DRP1, as cell death effectors has been demonstrated, and yet the activity of these mitochondrial dynamics components is not required for apoptosis in *C. elegans* (37). A second recent

report shows that stimulation of mitochondrial fission by Bax/Bak is not required for cytochrome *c* release, as antiapoptotic Bcl-2 inhibits this Bax/Bak-induced release, but not their effects on mitochondrial fission (268). In conclusion, it is still an open question as to whether mitochondrial fission is a key factor regulating apoptosis or whether it is only an epiphenomenon of programmed cell death.

VII. IMPLICATIONS IN HUMAN PATHOLOGY

In addition to the evidence that supports the relevance of mitochondrial dynamics proteins in cell function, mutations in genes encoding for some of these proteins are responsible for human disease. In this section we review the information recently gathered. We also analyze the potential implication of these proteins in metabolic diseases such as obesity and type 2 diabetes.

A. OPA1 Mutation: Autosomal Dominant Optic Atrophy

In 2000, two simultaneous studies reported OPA1 mutations in patients affected by autosomal dominant optic atrophy or ADOA (3, 76). ADOA occurs with an estimated prevalence that ranges from 1:12,000 to 1:50,000 (169, 226) and is the most common form of inherited optic neuropathy. ADOA is a genetically heterogeneous disease, and five loci have been linked to the disease (named OPA1 to OPA5) (226). Among these, the most common form of ADOA is caused by mutations in the *OPA1* gene.

ADOA is characterized by an insidious onset of visual impairment in early childhood with moderate to severe loss of visual acuity, temporal optic disc pallor, abnormalities of color vision, and cecocentral visual field scotoma (138, 152, 310). The disease is highly variable in clinical expression and shows incomplete penetrance in some families (138, 153, 309). Histopathological examination of donor eyes suggests that the fundamental pathology of ADOA is a primary degeneration of retinal ganglion cells followed by increasing atrophy of the optic nerve (152, 170).

According to the eOPA1 database, 117 *OPA1* gene mutations have been identified, which are basically family specific (95; http://lbbma.univ-angers.fr/eOPA1). OPA1 mutations largely consist of substitutions (66%), although deletions (28%) and insertions (6%) have also been reported (see reviews in Refs. 75, 226). Almost 50% of the mutations cause premature truncation of the OPA1 protein. The distribution of OPA1 mutations is depicted in Figure 13. Most of the mutations are detected in the GTPase domain (~41%). OPA1 mutants have also been identified in other regions: *1*) in the NH_2-terminal region of OPA1 encompassing the mitochondrial import sequence, the hydrophobic sequences and a coiled-coil region (12% of mutations); *2*) in the middle domain (33% of mutations); and *3*) in the GTPase effector domain or GED domain (14% of mutations).

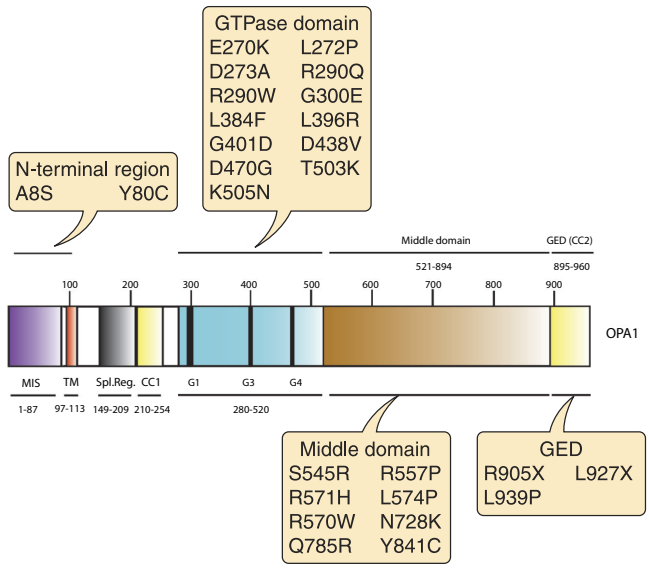


FIG. 13. Mutations in *OPA1* in human patients. Most of the missense mutations detected in autosomal dominant optic atrophy are shown. Most of the nonsense mutations or deletions are not shown. Mutations are grouped by domains affected.

In some cases, the pathogenesis of ADOA disease occurs as a result of haploinsufficiency. The major clinical data that support this concept are as follows: *1*) deletion of the entire *OPA1* gene or at aa residue 2 (Trp2X) (199, 233); *2*) almost 50% of the OPA1 mutants cause premature truncations of OPA1, a substantial number of them at the GTPase domain, and it is highly likely that they cause loss-of-function; and *3*) skin fibroblasts carrying OPA1 mutations show impaired oxidative phosphorylation and mitochondrial fusion (330). In keeping with these observations, OPA1 deletion induced by RNAi causes mitochondrial fragmentation, reduced mitochondrial membrane potential and cell respiration, disorganization of mitochondrial cristae, and apoptosis (59, 101, 116, 223). Haploinsufficiency of Opa1 in mice induced by in-frame deletion of 27 aa residues in the GTPase domain (in heterozygosis) exhibits degeneration of retinal ganglion cells and disorganized mitochondrial cristae of optic nerve axons (2). ENU-induced mutant mice carrying a protein-truncating nonsense mutation in Opa1 (Q285X, in hetozygosis) also show a 50% reduction of Opa1 protein, slow onset of degeneration in the optic nerve, and reduced visual function (71).

In addition to the presence of haploinsufficiency, some data indicate that ADOA develops as a consequence of a dominant negative mechanism. In support of this idea, several missense OPA1 mutations that ablate the consensus elements for GTP-binding have been reported

(G300E, G401D, K468E, or D470G) (23, 76, 167, 233; http://lbbma.univ-angers.fr/eOPA1), and GTPase mutants of OPA1 or of the yeast ortholog Mgm1p show a dominant negative effect in the presence of the wild-type protein (181, 267, 319). The dominant negative effect is due to the capacity of mutant OPA1 to oligomerize with wild-type proteins and, in this way, interfere with GTPase activity.

There are still relevant questions pending regarding the pathogenesis of ADOA. Why do OPA1 mutations selectively affect the retinal ganglion cell whereas other cell types in which OPA1 is substantially expressed remain preserved? Do mutations of OPA1 cause disease because of alterations in mitochondrial morphology or are they secondary to enhanced susceptibility to apoptosis? A deeper understanding of OPA1 biology is required to answer these and other relevant questions.

It has recently been found that OPA1 mutations cause dominant optic atrophy, with, in addition, progressive external ophthalmoplegia (PEO). This clinical phenotype has been defined as OPA1 "plus" syndrome. PEO is a clinical phenotype typically linked to mutations in genes involved in mitochondrial DNA maintenance such as *POLG1* (encoding the catalytic α-subunit of DNA polymerase γ), *POLG2* (encoding the accessory β-subunit of DNA polymerase γ), *PEO1* (encoding Twinkle), *TK2* (encoding thymidine kinase), *DGUOK* (encoding deoxyguanosine kinase), *TP* (encoding thymidine phosphorylase), *SLC25A4* (adenine nucleotide translocator 1 or Ant1), *SUCLA2*, *MPV17*, or *RRM2B* and is characterized by a reduction in mtDNA copy number (64). In keeping with this, patients with certain OPA1 mutations such as A357T, G439V, R445H, S545R, or V910D show multiple deletions in mitochondrial DNA in skeletal muscle (6, 140).

B. MFN2 Mutation: Charcot-Marie-Tooth Type 2A

In 2004, six missense MFN2 mutations were reported in seven large and ethnically diverse pedigrees affected by Charcot-Marie-Tooth neuropathy type 2A (CMT2A) (336). CMT2A shows an autosomal dominant inheritance pattern.

Charcot-Marie-Tooth (CMT) disease (also named hereditary motor and sensory neuropathies HMSN) is clinically characterized by weakness and distal muscle atrophy, predominantly of the lower extremities, and sensory loss. Additional signs of CMT are loss of deep tendon reflexes or foot deformities such as high-arched feet, flat feet, or hammertoes, and less frequently, cranial nerve involvement, scoliosis, vocal cord paresis, or glaucoma. Approximately 1 in 2,500 individuals is diagnosed with CMT, which makes this condition one of the most common hereditary diseases and the most common hereditary neuropathy (271).

CMT is a genetically and clinically heterogeneous disorder that is classified as demyelinating forms (which usually include CMT1, CMT3, and CMT4) or axonal form (also named CMT2). Demyelinating neuropathies such as CMT1 are characterized by a severely reduced motor nerve conduction velocity ($<$38 m/s). CMT2 is characterized by chronic axonal degeneration and regeneration, leading to steady loss of nerve fibers with normal or slightly reduced motor nerve conduction velocities ($\geq$38 m/s) (84, 85).

Mutations in MFN2 account for 20% of CMT2 cases (179), making this the most prevalent axonal form of CMT. CMT2 cases show high variability in clinical symptoms, and it has been reported that 25% of subjects within the families studied with an MFN2 mutation present very mild features (179). This has raised the necessity of performing molecular genetic testing for MFN2 in the presence of any signs of CMT even when normal findings have been recorded.

At the other extreme, MFN2 mutations have been detected in CMT2 families and associated with additional features such as spasticity (also referred as hereditary motor and sensory neuropathy type V, HMSN V) (332) or atrophy (HMSN VI) (335).

More than 40 mutant forms of MFN2 have been reported in CMT2A patients (57, 89, 165, 179, 307, 332, 335, 336). Most of the mutations described are missense. The distribution of mutants in the MFN2 protein is shown in Figure 14. Most are detected in the GTPase domain of MFN2 ($>$50%) (lying from aa residues 99 to 310). In addition, MFN2 mutants have been detected in some regions of MFN2: *1*) in the NH_2-terminal region, near or at the Ras-binding domain (aa residues 77 to 92) (5 mutations); *2*) in the region encompassing aa residues 357 to 424, i.e., in the vicinity or at the coiled-coil region 1 (406-433) (12 mutations); and *3*) in the COOH terminus, at the coiled-coil 2 region, facing the cytoplasmic site (4 mutations).

With regard to the ~23 mutations reported in the GTPase domain, it should be pointed out that none affects the consensus elements G1-G5, and in keeping with this view, some of the mutants analyzed, such as P251A or R280H, show normal GTP binding (21). Neither do mutations lying outside the GTPase domain show alterations in GTP binding (21).

Clinical studies indicate that some of the MFN2 mutations are found in more than one family within the same study or in separate studies. This is the case of mutations L76P, R94Q, R94W, T105M, T206I, R280H, R364W, or W740S. This observation indicates that mutational spots with high frequency are present in MFN2. In particular, mutations in aa residue 94 are abundant, thereby demonstrating that codon 94 is a hot spot.

Two large clinical studies have attempted to analyze a genotype/phenotype correlation in CMT2A patients, one

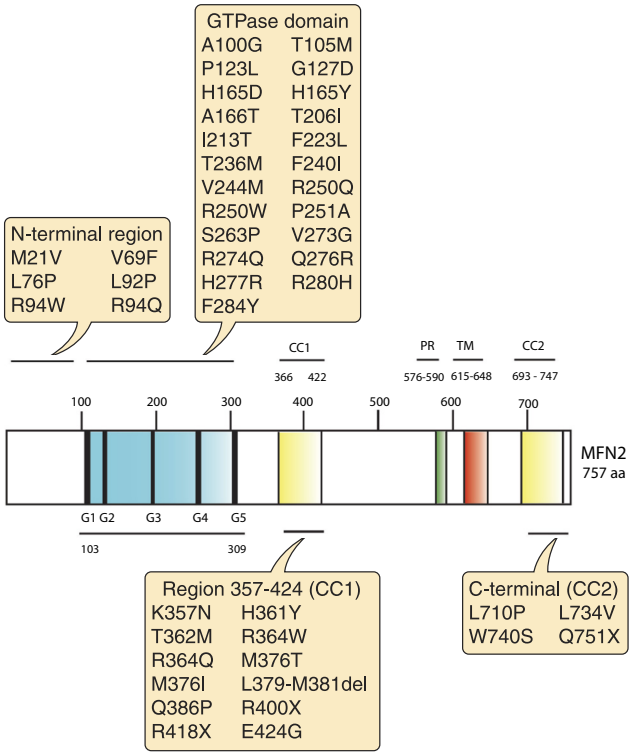


FIG. 14. Mutations in *Mfn2* in human patients. Most of the mutations detected in CMT2A are shown. Mutations are grouped by domains affected.

performed as a consortium of European, Israeli, and American institutions (307) and the second done in Korea (57). The studies coincide in the observation that two subgroups of CMT2 patients can be distinguished on the basis of the differences in age of onset and severity of disease. Thus some mutations are detected in patients showing an early onset of the disease (<10 years of age) and a strong clinical phenotype, whereas other MFN2 mutations are detected in patients with a later onset and a milder disease (57, 307). Although most of the mutations identified in the two studies differ, it is interesting to observe that both studies coincide that mutations such as L92P or R94W are linked to patients with early onset, and that in four unrelated families in the Korean study, the R364W mutation is also linked to early onset of disease. In contrast, M376T is detected in late-onset patients in both studies, and in the Korean study several families carrying the H165R mutation were also detected in a late-onset form of the disease. Furthermore, it is of interest that mutation R280H was identified in patients with late onset of disease (in 6 individuals from the same family) in the Korean study, whereas the same mutation was detected in two families of distinct origin (one from Italy and another from Belgium), one having early onset and the other showing late onset of disease. These data suggest that the environment or modulatory genes regulate the intensity of the phenotype. However, further studies with more extensive phenotyping in more families are needed to generate a more precise genotype/phenotype relationship in subjects with MFN2 mutations.

Given that the mutations of MFN2 responsible for CMT2A show autosomal dominant inheritance, they may show haploinsufficiency (loss-of-function) or a dominant gain-of-function. In this regard, the overexpression of some mutant forms of MFN2 (V69F, L76P, R94Q, P251A, R280H, or W740S) induces aggregation of mitochondria in cultured rat dorsal root ganglion neurons (21). This effect was not detected after overexpression of wild-type Mfn2; therefore, these data suggest a dominant gain-of-function (21). In keeping with this view, overexpression of some mutant forms of Mfn2 (L76P, R94W, T105M or W740S) causes abnormal aggregation of mitochondria in MEFs, whereas other mutant forms or wild-type Mfn2 do not produce mitochondrial aggregation (V69F, R94Q, P251A, R274Q, or R280H) (78). In this regard, transgenic mice have been generated to express a mutant form of Mfn2 with a presumed gain-of-function (T105M) specifically in motor neurons. These mice show a phenotype consistent with the clinical symptoms detected in CMT2A, such as hindlimb gait defects caused by inability to dorsiflex the hindpaws, defective mitochondrial distribution in motor neurons, reduced numbers of motor axons in the motor roots, and severe reduction of the anterior calf muscles (79).

In addition to the dominant effects displayed by some Mfn2 mutants, Mfn2 mutants such as R94Q, R94W, T105M, P251A, or R280H also show lack of mitochondrial fusion activity when assayed in double *Mfn* KO (i.e., for *Mfn1* and *Mfn2*) MEFs (78). In a separate study, MFN2 mutants (V69F, R94Q, P251A, R274Q, or R280H) have been shown to induce lower mitochondrial mobility in cultured neurons (21).

Interestingly, it has been reported that the defective mitochondrial fusion activity of Mfn2 mutants is rescued by expression of Mfn1, which is in keeping with the observation that Mfn1 physically associates with Mfn2 wild-type and CMT2A mutant forms. This interaction with Mfn1 may explain that human skin fibroblasts obtained from several CMT2A patients show no alterations in mitochondrial network or in mitochondrial ultrastructure or mitochondrial fusion (8, 193).

CMT2A is an axonopathy, and this is in keeping with the phenotype obtained in conditional *Mfn2* knockout mice. Thus ablation of Mfn2 specifically in cerebellum in mice causes neurodegeneration (52). Under these conditions, Purkinje cells show aberrant mitochondrial distribution and reduced dendritic outgrowth and spine formation (52). These data indicate that Mfn2 is essential to maintain neuron function. At present, the interaction between Mfn1 and mutations of Mfn2 and the capacity of Mfn1 to rescue function is the only mechanistic explanation available to explain the tissue-specific alterations in CMT2A patients.

Another conclusion drawn from the data available on CMT2A *MFN2* mutations is the considerable heterogeneity regarding the alterations in their biological properties. Thus some MFN2 mutants show gain-of-function, others show loss-of-function, while others seem to display both gain- and loss-of-function (21, 78).

C. GDAP1 Mutation: Charcot-Marie-Tooth Type 4A

In 2002, two simultaneous studies reported GDAP1 mutations in patients affected by CMT4A (24, 70), and it is currently accepted that the subtype CMT4A is the most frequent recessive form of CMT.

CMT4 is clinically similar to but more severe than CMT1 and has an early average age of onset. CMT4 is classically defined as a demyelinating form of the disease and is associated with segmental de- and remyelination, in contrast to CMT2, which shows axonal degeneration without demyelination (29, 287). However, analysis of the nerve conduction rates of subjects with GDAP1 mutations indicates that while some mutations show low nerve conduction rates (such as W31X, T117 frameshift, R120W, G262 frameshift, or G271R) (9, 24, 261), others are characterized by normal rates (E114 frameshift, Q163X, S194X, L223X, or R282C) (9, 32, 215, 253, 265). These data demonstrate that some of the GDAP1 mutations cause axonal loss rather than demyelinization. It is relevant to point out that mutations such as S194X or R161H are associated with low or normal nerve conduction rates in individuals from distinct families (9, 24, 215). Moreover, siblings within one family carrying the mutation R161H show a low nerve conduction rate or normal values (9).

The presence of axonopathy in patients affected by CMT4A has raised the question of whether the primary action of GDAP1 occurs in neurons, in Schwann cells, or in both cell types. In this regard, GDAP1 is expressed in myelinating Schwann cells and in neurons of the peripheral nervous system in rodents (218, 232), and there is some evidence indicating that *GDAP1* gene expression is much higher in the latter (232), and that Schwann cells in culture do not express GDAP1 (232). These observations suggest that neurons of the peripheral nervous system are the main cell type affected in this disease. However, more studies are required to demonstrate this point.

GDAP1 mutants show defective mitochondrial fission activity, although they localize in mitochondria after expression in COS-7 cells (218). Mutants R161H and R310Q are completely inactive with respect to the promotion of mitochondrial fragmentation, and mutants M116H, R120Q, or R282C show some residual effect on mitochondrial fragmentation although largely reduced compared with the activity of wild-type GDAP1 (218). Interestingly, the T157P mutation, originally described as a de novo dominant mutation in a sporadic case (61), has been reported to induce mitochondrial aggregation, thereby suggesting a potential gain-of-function (231).

D. DRP1 Mutation: Abnormal Brain Development

A report on a newborn girl with microcephaly, abnormal brain development, optic atrophy and hypoplasia, persistent lactic acidemia, and a mildly elevated plasma concentration of very-long-chain fatty acids was recently reported (317). The infant was born at term, and during the pregnancy, the mother noted diminished fetal movements. At 6 days of age, the newborn showed poor feeding, was mildly dysmorphic, with deep-set eyes, a pointed chin, and a head circumference below the 0.4 percentile. Magnetic resonance imaging of the brain revealed an abnormal gyral pattern in both frontal lobes that extended to the perisylvian areas and was associated with dysmyelination. In addition, the infant showed truncal hypotonia with little spontaneous movement and no tendon reflexes and showed no response to light stimulation (317). The disorder was lethal, and the patient died at the age of 37 days.

The patient also showed metabolic alterations that consisted of elevated blood lactate concentrations, elevated plasma alanine concentrations, and increased cerotic acid concentrations (a very-long-chain fatty acid) and an elevated ratio of cerotic acid to docosanoic acid concentrations. The patient also had elevated cerebrospinal fluid concentrations of lactate, alanine, pyruvate, and protein (317). These data suggest alterations in mitochondrial oxidation (oxidation of pyruvate) as well as in peroxisomal oxidation (oxidation of very-long-chain fatty acids). In keeping with these observations, fibroblasts obtained from the patient showed defective mitochondrial and peroxisomal fission. Genetic analysis revealed a heterozygous missense mutation in DRP1 (A395D), mapping to a conserved amino acid in the middle domain of the protein. This mutation behaves as a dominant form and causes the inhibition of both peroxisomal and mitochondrial fission after expression in fibroblasts (317). This inhibition could be explained by a possible defect in the initial oligomerization of DRP1 that is required to form the proper high-assembly structures on membranes (i.e., the spiral rings). This hypothetical role for the middle domain has been recently demonstrated in conventional dynamins (245).

In summary, the A395D DRP1 mutation causes a lethal disease that may reflect defects in mitochondrial or in peroxisomal function. The severity of the clinical traits suggests that mitochondria and peroxisomes are involved in the pathogenesis of the disease; however, this requires further experimental evidence.

E. Mutations in LETM1: The Wolf-Hirschhorn Syndrome

Up to this point, we have reviewed the information available on the mutations in genes known to promote mitochondrial fusion or fission and that cause human disease. As we mentioned in the initial sections of this review, we expect to identify additional proteins that participate in mitochondrial fusion or fission in mammalian cells. In addition, we will learn about proteins that modulate mitochondrial shape by performing functions other than mitochondrial fusion or fission. This relates to the topic of this specific section.

The Wolf-Hirschhorn syndrome (WHS) is a complex malformation disorder caused by the deletion of parts of the distal short arm of chromosome 4 and has an incidence of 1/50,000 live births (28). WHS is characterized by growth and mental retardation, congenital hypotonia, distinct facial appearance, congenital heart defects, midline defects, and seizures. An unknown number of genes contribute to the phenotype. A WHS critical region (WHSCR) has been localized to a 165-kb region between the loci D4S166 and D4S3327 (320). The 5′ end of the *LETM1* gene is located near 80 kb distal to the WHSCR and is deleted in all WHS patients with the full phenotype. One patient was reported to have a small interstitial deletion of 191.5 kb encompassing the WHSCR but excluding LETM1. This patient showed very mild affectation and no seizures, thereby suggesting that LETM1 is involved in some of the neuromuscular features of WHS (248).

LETM1 encodes a putative 83.5-kDa protein with a single transmembrane domain, two possible low-affinity EF-hand motifs (that probably bind Mg^{2+}), a leucine zipper, and several α-helical structures with high probabilities for forming coiled-coils (88). LETM1 is ubiquitously expressed. LETM1 is an inner mitochondrial membrane protein (80) and shows amino acid sequence identity with mitochondrial proteins from several species, including the putative protein MDM38/YOL027C from *S. cerevisiae*. Deletion of the gene in yeast causes mitochondrial dysfunction, increased cation contents (especially K^+), and swollen mitochondria (220). Thus Mdm38/Yol027 is proposed to be involved in organelle morphology and to participate in the K^+/H^+ exchange across the inner mitochondrial membrane (220). In keeping with these observations, LETM1 modulates mitochondrial shape in mammalian cells. Thus LETM1 downregulation causes nonmitochondrial cell death, DRP1-independent fragmentation of the mitochondrial network, mitochondrial swelling, and cristae disorganization (80, 293). Mitochondrial swelling is not a consequence of the disassembly of the respiratory chains (293). In fact, the defects in mitochondrial morphology caused by LETM1 loss-of-function can be restored by the normalization of K^+ fluxes across the inner membrane (80, 293). In all, these data highlight the importance of ion fluxes and LETM1 in the maintenance of normal mitochondrial shape and function. The detailed mechanisms of LETM1 function and identification of partners deserve special attention.

F. Defective Expression of Mitochondrial Dynamics Proteins: Implications in Obesity and Type 2 Diabetes

A central characteristic of type 2 diabetes is insulin resistance, which affects skeletal muscle (161, 234, 235, 297). There is substantial evidence indicating that insulin resistance precedes the development of type 2 diabetes (74, 190, 191) and that it plays a primary role in its pathogenesis. In fact, insulin resistance in the offspring of patients with type 2 diabetes has been shown to be the best predictor of the development of the disease (315).

The association between obesity and type 2 diabetes is also well documented in the literature, which is in keeping with the observation that an increase in fat mass is one of the major determinants of insulin resistance (178). A main feature of this condition is the reduced capacity of the muscle to properly oxidize substrates, glucose and lipids, during fasting conditions and after a meal. The switch between glucose and lipid oxidation, depending on energy requirements, is referred to as "metabolic flexibility" (162). Obese and type 2 diabetic subjects show metabolic inflexibility since they present a higher capacity to oxidize lipids in insulin-stimulated conditions, instead of switching towards glucose oxidation (93, 286).

Mitochondria, which are involved in the fine tuning of several cellular functions, also play a crucial role in the oxidation of fuels (20). Furthermore, skeletal muscle mitochondrial function is closely associated with insulin resistance. Mitochondrial number and size are reduced in the skeletal muscle of obese and type 2 diabetic patients compared with lean subjects (161, 297). The impairment of mitochondrial metabolism in muscle of type 2 diabetic patients is characterized by dysregulation of OXPHOS (161, 270) and by reduction in the expression of genes encoding distinct OXPHOS subunits (213, 230, 281).

In addition to type 2 diabetes, defective mitochondrial function in skeletal muscle has been reported in other insulin-resistant states (234, 235, 297). In skeletal muscle of nondiabetic individuals with a family history of type 2 diabetes, decreased expression of nuclear genes encoding proteins of oxidative phosphorylation has been described (213, 230), along with reduced in vivo oxidative phosphorylation (213, 235).

1. MFN2 is repressed in skeletal muscle in obesity

Studies of subtractive hybridization have permitted the identification of Mfn2 in rat skeletal muscle and the

detection of *Mfn2* repression in skeletal muscle of obese Zucker rats (16). This animal model of obesity shows a skeletal muscle metabolic profile characterized by reduced glucose uptake and glucose oxidation, altered partitioning of fatty acids that are incorporated into triglycerides or oxidized, insulin resistance and reduced oxygen consumption (67, 69, 156, 163, 301). Northern and Western blot assays have confirmed lower expression of *Mfn2* mRNA and protein in muscle of Zucker obese rats than in lean controls (16). In parallel to these observations, the extent of the mitochondrial network in skeletal muscle was estimated as the ratio between mitochondrial volume to unit of mitochondrial surface (17), measured by electron microscopy. These assays revealed a 25% reduction in the mitochondrial network in skeletal muscle of obese Zucker rats, which is parallel to the downregulation of the mitochondrial protein Mfn2 (16). This reduction was detected under conditions in which no differences were found in the total mitochondrial volume between control and obese groups, thereby indicating that the total mitochondrial mass was unaltered (16).

In keeping with the observations in obese Zucker rats, skeletal muscle of obese subjects also shows a reduced expression of *MFN2* mRNA compared with lean subjects (15, 16). The decrease in *MFN2* mRNA levels is found in both males and females (15), and under these conditions, the expression of MFN2 protein is also downregulated (16).

Weight reduction is one of the best approaches to ameliorate insulin sensitivity, and bariatric surgery has emerged as a potential therapy for diabetes (41). Biliopancreatic diversion (BPD) is a bariatric surgical technique characterized by massive weight loss caused mainly by lipid malabsorption (46, 113). BPD causes a net improvement in insulin sensitivity, long before the normalization of body weight (118). In addition, BPD regulates substrate oxidation, thereby modulating the expression of genes involved in lipid synthesis (110, 210) and oxidation (110, 209, 308) in both muscle and adipose tissue. In this regard, increased *MFN2* mRNA expression has been reported after BPD in skeletal muscle of morbidly obese subjects with normal glucose tolerance (208). Weight loss also causes a slight increase in the abundance of mRNA for citrate synthase or COX3 (208). The induction in MFN2 is not specific of the surgical procedure and, in fact, bariatric surgery performed by using the Roux-en-Y gastric bypass also shows a similar upregulation of *MFN2* (108).

In all, currently available data indicate that MFN2 is downregulated in skeletal muscle in human obesity and in obese Zucker rats, and this is accompanied by a reduction in mitochondrial size and in the extent of the mitochondrial network. The observations that *1*) obese Zucker rats show a primary defect that lies at the leptin receptor level rather than in mitochondria, and *2*) MFN2 is induced in response to massive weight loss in morbidly obese subjects, clearly indicate that upstream factors are responsible for Mfn2 dysregulation.

2. Defective MFN2 in skeletal muscle of type 2 diabetic subjects

Type 2 diabetic patients also show a reduction in *MFN2* expression in skeletal muscle compared with control subjects (15) (Table 5). MFN2 repression is detected in skeletal muscle of both obese or nonobese type 2 diabetic patients (15). It is unlikely that the dysregulation of this expression is a consequence of reduced insulin action as MFN2 expression in healthy, obese, or type 2 diabetic subjects is not altered in response to 3 h of hyperinsulinemia (150 mU/l) during euglycemic-hyperinsulinemic clamps (15), nor is the expression of this protein affected when cultured muscle cells are incubated in the presence of supramaximal insulin concentrations for up to 48 h (15).

Tumor necrosis factor (TNF)-α and interleukin-6 are crucial factors involved in insulin resistance. TNF-α contributes to obesity-related insulin resistance (134), and several animal models of obesity and insulin resistance show significantly higher levels of TNF-α mRNA and protein compared with their lean counterparts (124, 135, 136). Moreover, neutralization of TNF-α with a soluble TNF-α receptor-IgG fusion protein ameliorates insulin sensitivity (137). Interleukin-6 is produced by several cell types including macrophages, adipose cells, and skeletal muscle, and circulating levels are increased in type 2 diabetes (237, 242), thereby interfering with insulin signaling in muscle and hepatic cells (171, 250) and causing hepatic and muscle insulin resistance in humans and in mice (40, 166, 171, 250, 262, 284, 300). In this regard, our laboratory has obtained some evidence indicating that TNF-α or interleukin-6 inhibits Mfn2 gene expression in cells in culture (15) (Table 5). Whether these two factors

TABLE 5. *Conditions or agents that regulate Mfn2 expression in cells or tissues*

Mfn2	Reference Nos.
Reduced	
Human obesity (skeletal muscle)	15, 16
Human type 2 diabetes (skeletal muscle)	15
Obese Zucker rats (skeletal muscle)	16
TNF-α (NIH3T3 fibroblasts)	15
Interleukin-6 (human skeletal muscle cells)	15
Enhanced	
Human weight loss (skeletal muscle)	108, 208
Cold exposure (skeletal muscle and brown adipose tissue in rats)	278
β3-Adrenergic agonist (CL-316243) (skeletal muscle and brown adipose tissue in rats)	278
Exercise (skeletal muscle)	44

TNF, tumor necrosis factor.

play a regulatory in vivo role on Mfn2 expression awaits further demonstration.

At present, we do not have a global view of the expression of other proteins directly involved in mitochondrial fusion (MFN1, OPA1) or in fission (DRP1, FIS1) in muscle during obesity, in human diabetes, or in animal models of disease. However, it of interest that the expression of one of the mitochondrial proteases that is involved in OPA1 degradation, PARL, is also reduced in an animal model of diabetes. Thus the gene expression of Parl is reduced in skeletal muscle of diabetic *Psammomys obesus*, and exercise training normalizes the levels of Parl mRNA (313). In humans, a positive linear correlation was detected between PARL mRNA levels and insulin sensitivity, as assessed by glucose disposal during the hyperinsulinemic-euglycemic clamp and the polymorphic variant Leu262Val associated with increased plasma insulin concentrations, a surrogate of insulin sensitivity (313). Although the relationship between PARL and mitochondrial dynamics is not fully understood, present data allow us to speculate that mitochondrial fusion activity is a defective process under insulin-resistant states.

3. *Implications of muscle MFN2 repression in metabolism*

Several observations (discussed in sect. v) support the notion that MFN2 controls mitochondrial metabolism, namely, that Mfn2 loss-of-function reduces glucose oxidation, mitochondrial membrane potential, oxygen consumption, and mitochondrial proton leak in cultured cells (16, 50, 236). In this regard, the observation that both obesity and type 2 diabetes show reduced MFN2 expression and a reduced mitochondrial size could be highly relevant. Further studies in rodents after conditional recombinant ablation of Mfn2 are required to determine whether this protein contributes to metabolic disturbances linked to obesity or diabetes.

Two aspects are of special interest regarding MFN2. Does MFN2 loss-of-function enhance susceptibility to developing obesity? Or does MFN2 regulate insulin sensitivity?

As to the first question, a negative linear relationship between MFN2 mRNA levels and body mass index has been detected in control, obese, or type 2 diabetic patients regardless of sex (15). Such a relationship does not provide any evidence that MFN2 promotes obesity. However, Mfn2-depleted cells show a lower mitochondrial proton leak in the absence of alterations in uncoupled respiration, which suggests enhanced bioenergetics efficiency (16). Extrapolation of these data to the in vivo animal allows us to speculate that Mfn2 loss-of-function contributes to obesity by promoting a more efficient energetic state, which would reduce basal energy expenditure and enhance energy storage, mainly in the form of fat. In agreement with a potential role of MFN2 in some of the metabolic alterations associated with obesity, our group has reported that skeletal muscle of obese Zucker rats shows a reduced expression of some subunits of complexes I, II, III, and V, in the absence of changes in the abundance of complex IV subunits or porin (236). This pattern of alterations in the content of subunits of the OXPHOS system is very similar to that detected in Mfn2-knockdown muscle cells (236).

As regards the connection between MFN2 and insulin sensitivity, we have detected a positive correlation between MFN2 expression in skeletal muscle and insulin sensitivity, assessed by glucose disposal rates measured by the insulin clamp in healthy control subjects, and in obese or type 2 diabetic patients (15). In addition, a positive correlation between MFN2 levels and insulin sensitivity, measured by the glucose clamp, has been found in morbidly obese subjects and after BPD (15, 208).

4. *Conditions characterized by increased energy expenditure also show higher MFN2 expression*

If MFN2 plays a regulatory role in mitochondrial bioenergetics in vivo, it should be upregulated under conditions linked to enhanced energy expenditure. Available data indicate that, indeed, Mfn2 is induced in response to conditions known to enhance energy expenditure (Table 5). Thus rats exposed for two days to cold show a marked and specific enhancement in *Mfn2* gene expression in brown adipose tissue and in skeletal muscle (278). Furthermore, administration of CL-316243 (a β_3-adrenergic agonist) enhances *Mfn2* expression in these two tissues (278). It should be emphasized that the expression of Mfn2 is markedly enhanced under conditions in which genes encoding other mitochondrial proteins, Cox2, Atp5a1, or cytochrome *c*, show minor increases (278). The stimulation of *Mfn2* gene expression in skeletal muscle and brown adipose tissue under conditions of enhanced energy expenditure may play a relevant role in the adaptive regulation of the mitochondrial metabolism to maintain energy homeostasis.

Chronic exercise is another condition known to induce mitochondrial biogenesis in skeletal muscle and enhance mitochondrial function (96, 111, 131). In this regard, a single bout of exercise induces MFN2 and MFN1 gene expression in skeletal muscle of healthy subjects (44).

5. *Mfn2 is a key target of the PGC-1α regulatory pathway*

Exposure to cold, treatment with CL-316243, or exercise enhance Ppargc1a (PGC-1α) expression in tissues (33, 238, 244). These observations have led to the analysis of whether PGC-1α regulates Mfn2 transcription. PPARGC1A (PGC-1α) overexpression markedly enhances Mfn2 mRNA and protein levels in cultured muscle cells,

under conditions in which other genes typically induced by PGC-1α, such as Atp5a1, Cox2, or cytochrome *c*, are more moderately enhanced (278). PGC-1α also stimulates the transcriptional activity of the human MFN2 gene promoter (2 kb) after transfection in several cell types, and mapping analysis of the promoter has identified a region localized between −432/−352, which is critical for conferring the capacity to respond to PGC-1α (278). In this region, three putative binding boxes for nuclear hormone receptors conserved in human and mouse MFN2 promoters have been identified, and cancellation of the middle box or box 2 (localized at −410/−405) completely blocks the response to PGC-1α (278). Chromatin immunoprecipitation and transfection analyses have indicated that this specific region of the MFN2 promoter binds to and is activated by the nuclear hormone receptor ERRα and is further coactivated by PGC-1α (278).

In all, PGC-1α stimulates MFN2 expression through the operation of a transcriptional mechanism that involves the activation of the MFN2 promoter, which requires the integrity of an ERRα-binding element. This conclusion is based on a number of observations, namely: *1*) ERRα activates the transcriptional activity of the MFN2 promoter, and the effects are synergic with those of PGC-1α; *2*) cancellation of the ERRα-binding element blocks or diminishes the effect of PGC-1 on promoter activity; *3*) ERRα binds in vitro to the ERRα-binding element −410/−405 and in vivo to a MFN2 promoter fragment centered around the ERRα-binding element; and *4*) PGC-1α binds in vivo the MFN2 promoter in a region centered around the ERRα-binding element (278).

In our opinion, *MFN2* is not simply another mitochondrial protein-encoded nuclear gene that is upregulated by the nuclear coactivator PGC-1α but a component of intracellular signaling machinery that regulates cell metabolism. In this regard, there are several lines of evidence indicating that the effect of PGC-1α on mitochondrial membrane potential requires normal expression of Mfn2. Thus Mfn2 loss-of-function reduces the effect of PGC-1α on mitochondrial membrane potential in 10T1/2 fibroblasts (278). Similarly, the stimulatory effect of PGC-1α on mitochondrial membrane potential is reduced in *Mfn2* −/− mouse embryonic fibroblast cells (278). Under all these conditions, the effect of PGC-1α on mitochondrial biogenesis is not altered in Mfn2-depleted cells (278). These data suggest that the effects of PGC-1α on mitochondrial energization require or are partly mediated by Mfn2.

At present, we favor the existence of a regulatory pathway that drives mitochondrial metabolism and that is defined by PGC-1α, ERRα, and MFN2. The pathway is characterized by a stimulatory action of PGC-1α on the transcription of MFN2, via coactivation of ERRα (Fig. 15). Support of this hypothesis comes from several studies as reviewed above, i.e., *1*) PGC-1α activates Mfn2 expression in cells, *2*) the mechanisms by which PGC-1α stimulates this expression are dependent on intact ERRα binding in the MFN2 promoter; and *3*) the full metabolic effects of PGC-1α are detected only in the presence of normal Mfn2 activity.

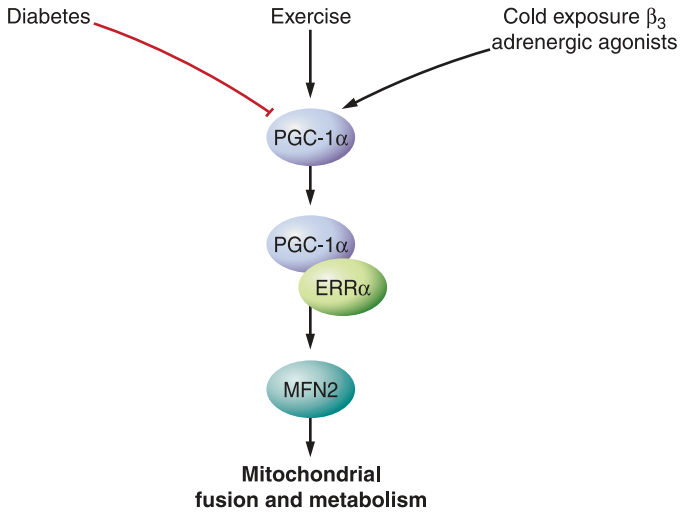


FIG. 15. PGC-1α, ERRα, and Mfn2 define a mitochondrial regulatory pathway. Mfn2 is induced by PGC-1α and ERRα in muscle cells. The participation of PGC-1α permits the induction of Mfn2 in response to exercise, cold exposure, or β_3-adrenergic agonists. Mfn2 drives mitochondrial fusion as well as mitochondrial metabolism, and consequently, Mfn2 may play a key role in some of the biological effects of PGC-1α on mitochondrial function.

Further evidence that MFN2 constitutes a regulatory pathway in conjunction with PGC-1α and ERRα comes from studies performed in heterozygous *Mfn2* knockout mice (homozygous knockout are not viable) subjected to cold for 48 h. These animals show a twofold greater induction of PGC-1α expression in skeletal muscle compared with wild-type mice (278), which is accompanied by an enhanced induction of *Mfn2* expression (278). These data suggest a possible Mfn2-induced homeostatic process to regulate PGC-1α. The mechanism does not involve a direct effect of Mfn2 because RNAi-induced repression of Mfn2 does not alter PGC-1α expression in C2C12 muscle cells (M. Liesa and A. Zorzano, unpublished observations).

The regulatory pathway formed by PGC-1α, ERRα, and MFN2 may be altered in insulin-resistant conditions and particularly in type 2 diabetes. In agreement with this, it has been reported that MFN2 and PGC-1α expression is deficient in type 2 diabetic subjects and in insulin-resistant subjects (15, 213, 230). On the basis of the relevant role played by PGC-1α and mitochondria in cell metabolism, alterations in the PGC-1α/MFN2 axis may confer susceptibility to the development of insulin resistance and diabetes mellitus.

VIII. CONCLUSIONS AND PERSPECTIVES

During the last few years, a significant amount of relevant data has been obtained on the identification of proteins involved in mitochondrial dynamics in mammalian cells and on their function. We still await the identification of additional proteins involved in mitochondrial distribution and shape, as well as in mitochondrial fusion and fission in mammalian cells. As to the proteins already identified, we have learned some basic lessons, namely, *1*) that genes encoding some of these proteins cause human diseases such as in the case of Charcot-Marie Tooth type 2A or type 4A or Kjer disease (autosomal dominant optic atrophy) and *2*) that mitochondrial dynamics is intimately related to mitochondrial function and cellular requirements in mammalian cells. In this regard, we know that some of the proteins involved in mitochondrial dynamics participate in central biological processes such as cellular metabolism, apoptosis, or the maintenance of mitochondrial DNA, although the specific mechanisms involved are poorly understood. A defective mitochondrial function has been invoked in many diverse complex disorders, such as obesity, type 1 or type 2 diabetes, and Parkinson's or Alzheimer's disease, and it is tempting to speculate that mitochondrial dynamics proteins are involved in the alterations in mitochondrial function that underlie these complex disorders. For these reasons, the study of mitochondrial dynamics offers an exciting and challenging field of study. If mitochondrial dynamics proteins are involved in complex diseases, the possibility of using them as drug targets will become relevant for biotech companies, especially for those proteins that are in the outer mitochondrial membrane oriented towards the cytosol and which, therefore, show more feasibility in drug discovery.

ACKNOWLEDGMENTS

We thank Tanya Yates for editorial support.

Address for reprint requests and other correspondence: A. Zorzano, Institute for Research in Biomedicine, C/Baldiri Reixac 10, 08028 Barcelona, Spain (e-mail: antonio.zorzano@irbbarcelona.org).

GRANTS

This study was supported by Ministerio de Educación y Cultura Grants SAF2005-00445 and SAF2008- and by Generalitat de Catalunya and CIBERDEM (Instituto de Salud Carlos III) Grant 2005SGR00947. M. Liesa was the recipient of a predoctoral fellowship from the Ministerio de Educación y Cultura, Spain. A. Zorzano is the recipient of a Science Intensification Award from the University of Barcelona.

REFERENCES

1. **Akepati VR, Muller EC, Otto A, Strauss HM, Portwich M, Alexander C.** Characterization of OPA1 isoforms isolated from mouse tissues. *J Neurochem* 106: 372–383, 2008.
2. **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.** A splice site mutation in the murine Opa1 gene features pathology of autosomal dominant optic atrophy. *Brain* 130: 1029–1042, 2007.
3. **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.** OPA1, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28. *Nat Genet* 26: 211–215, 2000.
4. **Alirol E, James D, Huber D, Marchetto A, Vergani L, Martinou JC, Scorrano L.** The mitochondrial fission protein hFis1 requires the endoplasmic reticulum gateway to induce apoptosis. *Mol Biol Cell* 17: 4593–4605, 2006.
5. **Altmann R.** *Die elementarorganismen und ihre beziehungen zu den zellen*. Leipzig, Germany: Viet, 1890.
6. **Amati-Bonneau P, Valentino ML, Reynier P, Gallardo ME, Bornstein B, Boissiere A, Campos Y, Rivera H, de la Aleja JG, Carroccia R, Iommarini L, Labauge P, Figarella-Branger D, Marcorelles P, Furby A, Beauvais K, Letournel F, Liguori R, La Morgia C, Montagna P, Liguori M, Zanna C, Rugolo M, Cossarizza A, Wissinger B, Verny C, Schwarzenbacher R, Martin MA, Arenas J, Ayuso C, Garesse R, Lenaers G, Bonneau D, Carelli V.** OPA1 mutations induce mitochondrial DNA instability and optic atrophy "plus" phenotypes. *Brain* 131: 338–351, 2008.
7. **Amchenkova AA, Bakeeva LE, Chentsov YS, Skulachev VP, Zorov DB.** Coupling membranes as energy-transmitting cables. I. Filamentous mitochondria in fibroblasts and mitochondrial clusters in cardiomyocytes. *J Cell Biol* 107: 481–495, 1988.
8. **Amiott EA, Lott P, Soto J, Kang PB, McCaffery JM, DiMauro S, Abel ED, Flanigan KM, Lawson VH, Shaw JM.** Mitochondrial fusion and function in Charcot-Marie-Tooth type 2A patient fibroblasts with mitofusin 2 mutations. *Exp Neurol* 211: 115–127, 2008.
9. **Ammar N, Nelis E, Merlini L, Barisic N, Amouri R, Ceuterick C, Martin JJ, Timmerman V, Hentati F, De Jonghe P.** Identification of novel GDAP1 mutations causing autosomal recessive Charcot-Marie-Tooth disease. *Neuromuscul Disord* 13: 720–728, 2003.
10. **Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJ, Staden R, Young IG.** Sequence and organization of the human mitochondrial genome. *Nature* 290: 457–465, 1981.
11. **Anesti V, Scorrano L.** The relationship between mitochondrial shape and function and the cytoskeleton. *Biochim Biophys Acta* 1757: 692–699, 2006.
12. **Arnoult D, Grodet A, Lee YJ, Estaquier J, Blackstone C.** 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, 2005.
13. **Arnoult D, Rismanchi N, Grodet A, Roberts RG, Seeburg DP, Estaquier J, Sheng M, Blackstone C.** Bax/Bak-dependent release of DDP/TIMM8a promotes Drp1-mediated mitochondrial fission and mitoptosis during programmed cell death. *Curr Biol* 15: 2112–2118, 2005.
14. **Asano M, Wakabayashi T, Ishikawa K, Kishimoto H.** Induction of megamitochondria in mouse hepatocytes by nialamide. *J Electron Microsc* 26: 141–144, 1977.
15. **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, 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.
16. **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, 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.

17. **Baddeley AJ, Gundersen HJ, Cruz-Orive LM.** Estimation of surface area from vertical sections. *J Microsc* 142: 259–276, 1986.
18. **Bakeeva LE, Chentsov YS, Skulachev VP.** Mitochondrial framework (reticulum mitochondriale) in rat diaphragm muscle. *Biochim Biophys Acta* 501: 349–369, 1978.
19. **Bakeeva LE, Chentsov YS, Skulachev VP.** Ontogenesis of mitochondrial reticulum in rat diaphragm muscle. *Eur J Cell Biol* 25: 175–181, 1981.
20. **Balaban RS.** Regulation of oxidative phosphorylation in the mammalian cell. *Am J Physiol Cell Physiol* 258: C377–C389, 1990.
21. **Baloh RH, Schmidt RE, Pestronk A, Milbrandt J.** Altered axonal mitochondrial transport in the pathogenesis of Charcot-Marie-Tooth disease from mitofusin 2 mutations. *J Neurosci* 27: 422–430, 2007.
22. **Baricault L, Segui B, Guegand L, Olichon A, Valette A, Larminat F, Lenaers G.** OPA1 cleavage depends on decreased mitochondrial ATP level and bivalent metals. *Exp Cell Res* 313: 3800–3808, 2007.
23. **Baris O, Delettre C, Amati-Bonneau P, Surget MO, Charlin JF, Catier A, Derieux L, Guyomard JL, Dollfus H, Jonveaux P, Ayuso C, Maumenee I, Lorenz B, Mohammed S, Tourmen Y, Bonneau D, Malthiery Y, Hamel C, Reynier P.** Fourteen novel OPA1 mutations in autosomal dominant optic atrophy including two de novo mutations in sporadic optic atrophy. *Hum Mutat* 21: 656, 2003.
24. **Baxter RV, Ben Othmane K, Rochelle JM, Stajich JE, Hulette C, Dew-Knight S, Hentati F, Ben Hamida M, Bel S, Stenger JE, Gilbert JR, Pericak-Vance MA, Vance JM.** Ganglioside-induced differentiation-associated protein-1 is mutant in Charcot-Marie-Tooth disease type 4A/8q21. *Nat Genet* 30: 21–22, 2002.
25. **Benard G, Bellance N, James D, Parrone P, Fernandez H, Letellier T, Rossignol R.** Mitochondrial bioenergetics and structural network organization. *J Cell Sci* 120: 838–848, 2007.
26. **Benda C.** Ueber dier Spermatogenese de Verbebraten und höherer Evertebraten, II. Theil: Die Histiogenese der Spermien. *Arch Anat Physiol* 73: 393–398, 1898.
27. **Bereiter-Hahn J, Voth M.** Dynamics of mitochondria in living cells: shape changes, dislocations, fusion, and fission of mitochondria. *Microsc Res Tech* 27: 198–219, 1994.
28. **Bergemann AD, Cole F, Hirschhorn K.** The etiology of Wolf-Hirschhorn syndrome. *Trends Genet* 21: 188–195, 2005.
29. **Berger P, Young P, Suter U.** Molecular cell biology of Charcot-Marie-Tooth disease. *Neurogenetics* 4: 1–15, 2002.
30. **Bibb MJ, Van Etten RA, Wright CT, Walberg MW, Clayton DA.** Sequence and gene organization of mouse mitochondrial DNA. *Cell* 26: 167–180, 1981.
31. **Bleazard W, McCaffery JM, King EJ, Bale S, Mozdy A, Tieu Q, Nunnari J, Shaw JM.** The dynamin-related GTPase Dnm1 regulates mitochondrial fission in yeast. *Nat Cell Biol* 1: 298–304, 1999.
32. **Boerkoel CF, Takashima H, Nakagawa M, Izumo S, Armstrong D, Butler I, Mancias P, Papasozomenos SC, Stern LZ, Lupski JR.** CMT4A: identification of a Hispanic GDAP1 founder mutation. *Ann Neurol* 53: 400–405, 2003.
33. **Boss O, Bachman E, Vidal-Puig A, Zhang CY, Peroni O, Lowell BB.** Role of the beta(3)-adrenergic receptor and/or a putative beta(4)-adrenergic receptor on the expression of uncoupling proteins and peroxisome proliferator-activated receptor-gamma coactivator-1. *Biochem Biophys Res Commun* 261: 870–876, 1999.
34. **Bourne HR, Sanders DA, McCormick F.** The GTPase superfamily: a conserved switch for diverse cell functions. *Nature* 348: 125–132, 1990.
35. **Bourne HR, Sanders DA, McCormick F.** The GTPase superfamily: conserved structure and molecular mechanism. *Nature* 349: 117–127, 1991.
36. **Brandt JT, Martin AP, Lucas FV, Vorbeck ML.** Structure of rat-liver mitochondria: re-evaluation. *Biochem Biophys Res Commun* 59: 1097–1103, 1974.
37. **Breckenridge DG, Kang BH, Kokel D, Mitani S, Staehelin LA, Xue D.** *Caenorhabditis elegans* drp-1 and fis-2 regulate distinct cell-death execution pathways downstream of ced-3 and independent of ced-9. *Mol Cell* 31: 586–597, 2008.
38. **Breckenridge DG, Stojanovic M, Marcellus RC, Shore GC.** Caspase cleavage product of BAP31 induces mitochondrial fission through endoplasmic reticulum calcium signals, enhancing cytochrome *c* release to the cytosol. *J Cell Biol* 160: 1115–1127, 2003.
39. **Brooks C, Wei Q, Feng L, Dong G, Tao Y, Mei L, Xie ZJ, Dong Z.** Bak regulates mitochondrial morphology and pathology during apoptosis by interacting with mitofusins. *Proc Natl Acad Sci USA* 104: 11649–11654, 2007.
40. **Bruce CR, Dyck DJ.** Cytokine regulation of skeletal muscle fatty acid metabolism: effect of interleukin-6 and tumor necrosis factor-alpha. *Am J Physiol Endocrinol Metab* 287: E616–E621, 2004.
41. **Buchwald H, Avidor Y, Braunwald E, Jensen MD, Pories W, Fahrbach K, Schoelles K.** Bariatric surgery: a systematic review and meta-analysis. *JAMA* 292: 1724–1737, 2004.
42. **Burgess SM, Delannoy M, Jensen RE.** MMM1 encodes a mitochondrial outer membrane protein essential for establishing and maintaining the structure of yeast mitochondria. *J Cell Biol* 126: 1375–1391, 1994.
43. **Campello S, Lacalle RA, Bettella M, Manes S, Scorrano L, Viola A.** Orchestration of lymphocyte chemotaxis by mitochondrial dynamics. *J Exp Med* 203: 2879–2886, 2006.
44. **Cartoni R, Leger B, Hock MB, Praz M, Crettenand A, Pich S, Ziltener JL, Luthi F, Deriaz O, Zorzano A, Gobelet C, Kralli A, Russell AP.** Mitofusins 1/2 and ERRalpha expression are increased in human skeletal muscle after physical exercise. *J Physiol* 567: 349–358, 2005.
45. **Cassidy-Stone A, Chipuk JE, Ingerman E, Song C, Yoo C, Kuwana T, Kurth MJ, Shaw JT, Hinshaw JE, Green DR, Nunnari J.** Chemical inhibition of the mitochondrial division dynamin reveals its role in Bax/Bak-dependent mitochondrial outer membrane permeabilization. *Dev Cell* 14: 193–204, 2008.
46. **Castagneto M, De Gaetano A, Mingrone G, Tacchino R, Nanni G, Capristo E, Benedetti G, Tataranni PA, Greco AV.** Normalization of insulin sensitivity in the obese patient after stable weight reduction with biliopancreatic diversion. *Obes Surg* 4: 161–168, 1994.
47. **Cereghetti GM, Stangherlin A, Martins dB, Chang CR, Blackstone C, Bernardi P, Scorrano L.** Dephosphorylation by calcineurin regulates translocation of Drp1 to mitochondria. *Proc Natl Acad Sci USA* 105: 15803–15808, 2008.
48. **Chang CR, Blackstone C.** Cyclic AMP-dependent protein kinase phosphorylation of Drp1 regulates its GTPase activity and mitochondrial morphology. *J Biol Chem* 282: 21583–21587, 2007.
49. **Chang DT, Reynolds IJ.** Differences in mitochondrial movement and morphology in young and mature primary cortical neurons in culture. *Neuroscience* 141: 727–736, 2006.
50. **Chen H, Chomyn A, Chan DC.** Disruption of fusion results in mitochondrial heterogeneity and dysfunction. *J Biol Chem* 280: 26185–26192, 2005.
51. **Chen H, Detmer SA, Ewald AJ, Griffin EE, Fraser SE, Chan DC.** Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. *J Cell Biol* 160: 189–200, 2003.
52. **Chen H, McCaffery JM, Chan DC.** Mitochondrial fusion protects against neurodegeneration in the cerebellum. *Cell* 130: 548–562, 2007.
53. **Chen KH, Guo X, Ma D, Guo Y, Li Q, Yang D, Li P, Qiu X, Wen S, Xiao RP, Tang J.** Dysregulation of HSG triggers vascular proliferative disorders. *Nat Cell Biol* 6: 872–883, 2004.
54. **Chen P, Harcum SW.** Differential display identifies genes in Chinese hamster ovary cells sensitive to elevated ammonium. *Appl Biochem Biotechnol* 141: 349–359, 2007.
55. **Choi SY, Huang P, Jenkins GM, Chan DC, Schiller J, Frohman MA.** A common lipid links Mfn-mediated mitochondrial fusion and SNARE-regulated exocytosis. *Nat Cell Biol* 8: 1255–1262, 2006.
56. **Chung JG, Yeh KT, Wu SL, Hsu NY, Chen GW, Yeh YW, Ho HC.** Novel transmembrane GTPase of non-small cell lung cancer identified by mRNA differential display. *Cancer Res* 61: 8873–8879, 2001.
57. **Chung KW, Kim SB, Park KD, Choi KG, Lee JH, Eun HW, Suh JS, Hwang JH, Kim WK, Seo BC, Kim SH, Son IH, Kim SM, Sunwoo IN, Choi BO.** Early onset severe and late-onset mild Charcot-Marie-Tooth disease with mitofusin 2 (MFN2) mutations. *Brain* 129: 2103–2118, 2006.

58. **Chung S, Dzeja PP, Faustino RS, Perez-Terzic C, Behfar A, 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.
59. **Cipolat S, Martins dB, Dal Zilio B, Scorrano L.** OPA1 requires mitofusin 1 to promote mitochondrial fusion. *Proc Natl Acad Sci USA* 101: 15927–15932, 2004.
60. **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.** Mitochondrial rhomboid PARL regulates cytochrome *c* release during apoptosis via OPA1-dependent cristae remodeling. *Cell* 126: 163–175, 2006.
61. **Claramunt R, Pedrola L, Sevilla T, Lopez dM, Berciano J, Cuesta A, Sanchez-Navarro B, Millan JM, Saifi GM, Lupski JR, Vilchez JJ, Espinos C, Palau F.** Genetics of Charcot-Marie-Tooth disease type 4A: mutations, inheritance, phenotypic variability, and founder effect. *J Med Genet* 42: 358–365, 2005.
62. **Cohen MM, Leboucher GP, Livnat-Levanon N, Glickman MH, Weissman AM.** Ubiquitin-proteasome-dependent degradation of a mitofusin, a critical regulator of mitochondrial fusion. *Mol Biol Cell* 19: 2457–2464, 2008.
63. **Collins TJ, Berridge MJ, Lipp P, Bootman MD.** Mitochondria are morphologically and functionally heterogeneous within cells. *EMBO J* 21: 1616–1627, 2002.
64. **Copeland WC.** Inherited mitochondrial diseases of DNA replication. *Annu Rev Med* 59: 131–146, 2008.
65. **Cortese JD.** Stimulation of rat liver mitochondrial fusion by an outer membrane-derived aluminum fluoride-sensitive protein fraction. *Exp Cell Res* 240: 122–133, 1998.
66. **Cortese JD, Voglino LA, Hackenbrock CR.** Novel fluorescence membrane fusion assays reveal GTP-dependent fusogenic properties of outer mitochondrial membrane-derived proteins. *Biochim Biophys Acta* 1371: 185–198, 1998.
67. **Crettaz M, Prentki M, Zaninetti D, Jeanrenaud B.** Insulin resistance in soleus muscle from obese Zucker rats. Involvement of several defective sites. *Biochem J* 186: 525–534, 1980.
68. **Cribbs JT, Strack S.** Reversible phosphorylation of Drp1 by cyclic AMP-dependent protein kinase and calcineurin regulates mitochondrial fission and cell death. *EMBO Rep* 8: 939–944, 2007.
69. **Cuendet GS, Loten EG, Jeanrenaud B, Renold AE.** Decreased basal, noninsulin-stimulated glucose uptake and metabolism by skeletal soleus muscle isolated from obese-hyperglycemic (*ob/ob*) mice. *J Clin Invest* 58: 1078–1088, 1976.
70. **Cuesta A, Pedrola L, Sevilla T, Garcia-Planells J, Chumillas MJ, Mayordomo F, LeGuern E, Marin I, Vilchez JJ, Palau F.** The gene encoding ganglioside-induced differentiation-associated protein 1 is mutated in axonal Charcot-Marie-Tooth type 4A disease. *Nat Genet* 30: 22–25, 2002.
71. **Davies VJ, Hollins AJ, Piechota MJ, Yip W, Davies JR, White KE, Nicols PP, Boulton ME, 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.
72. **De Brito OM, Scorrano L.** Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature* 456: 605–610, 2008.
73. **De Vos KJ, Allan VJ, Grierson AJ, Sheetz MP.** Mitochondrial function and actin regulate dynamin-related protein 1-dependent mitochondrial fission. *Curr Biol* 15: 678–683, 2005.
74. **DeFronzo RA, Bonadonna RC, Ferrannini E.** Pathogenesis of NIDDM. A balanced overview. *Diabetes Care* 15: 318–368, 1992.
75. **Delettre C, Griffoin JM, Kaplan J, Dollfus H, Lorenz B, Faivre L, Lenaers G, Belenguer P, Hamel CP.** Mutation spectrum and splicing variants in the OPA1 gene. *Hum Genet* 109: 584–591, 2001.
76. **Delettre C, Lenaers G, Griffoin JM, Gigarel N, Lorenzo C, Belenguer P, Pelloquin L, Grosgeorge J, Turc-Carel C, Perret E, Astarie-Dequeker C, Lasquellec L, Arnaud B, Ducommun B, Kaplan J, Hamel CP.** Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. *Nat Genet* 26: 207–210, 2000.
77. **Delivani P, Adrain C, Taylor RC, Duriez PJ, Martin SJ.** Role for CED-9 and Egl-1 as regulators of mitochondrial fission and fusion dynamics. *Mol Cell* 21: 761–773, 2006.
78. **Detmer SA, Chan DC.** Complementation between mouse Mfn1 and Mfn2 protects mitochondrial fusion defects caused by CMT2A disease mutations. *J Cell Biol* 176: 405–414, 2007.
79. **Detmer SA, Vande VC, Cleveland DW, Chan DC.** Hindlimb gait defects due to motor axon loss and reduced distal muscles in a transgenic mouse model of Charcot-Marie-Tooth type 2A. *Hum Mol Genet* 17: 367–375, 2008.
80. **Dimmer KS, Navoni F, Casarin A, Trevisson E, Endele S, Winterpacht A, Salviati L, Scorrano L.** LETM1, deleted in Wolf-Hirschhorn syndrome, is required for normal mitochondrial morphology and cellular viability. *Hum Mol Genet* 17: 201–214, 2008.
81. **Dohm JA, Lee SJ, Hardwick JM, Hill RB, Gittis AG.** Cytosolic domain of the human mitochondrial fission protein fis1 adopts a TPR fold. *Proteins* 54: 153–156, 2004.
82. **Duvezin-Caubet S, Jagasia R, Wagener J, Hofmann S, Trifunovic A, Hansson A, Chomyn A, Bauer MF, Attardi G, Larsson NG, Neupert W, Reichert AS.** Proteolytic processing of OPA1 links mitochondrial dysfunction to alterations in mitochondrial morphology. *J Biol Chem* 281: 37972–37979, 2006.
83. **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.** OPA1 processing reconstituted in yeast depends on the subunit composition of the m-AAA protease in mitochondria. *Mol Biol Cell* 18: 3582–3590, 2007.
84. **Dyck PJ, Lambert EH.** Lower motor and primary sensory neuron diseases with peroneal muscular atrophy. I. Neurologic, genetic, and electrophysiologic findings in hereditary polyneuropathies. *Arch Neurol* 18: 603–618, 1968.
85. **Dyck PJ, Lambert EH.** Lower motor and primary sensory neuron diseases with peroneal muscular atrophy. II. Neurologic, genetic, and electrophysiologic findings in various neuronal degenerations. *Arch Neurol* 18: 619–625, 1968.
86. **Dzau VJ, Braun-Dullaeus RC, Sedding DG.** Vascular proliferation and atherosclerosis: new perspectives and therapeutic strategies. *Nat Med* 8: 1249–1256, 2002.
87. **Eiberg H, Kjer B, Kjer P, Rosenberg T.** Dominant optic atrophy (OPA1) mapped to chromosome 3q region. I. Linkage analysis. *Hum Mol Genet* 3: 977–980, 1994.
88. **Endele S, Fuhry M, Pak SJ, Zabel BU, 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.
89. **Engelfried K, Vorgerd M, Hagedorn M, Haas G, Gilles J, Epplen JT, Meins M.** Charcot-Marie-Tooth neuropathy type 2A: novel mutations in the mitofusin 2 gene (MFN2). *BMC Med Genet* 7: 53, 2006.
90. **Escobar-Henriques M, Westermann B, Langer T.** Regulation of mitochondrial fusion by the F-box protein Mdm30 involves proteasome-independent turnover of Fzo1. *J Cell Biol* 173: 645–650, 2006.
91. **Eura Y, Ishihara N, Oka T, Mihara K.** Identification of a novel protein that regulates mitochondrial fusion by modulating mitofusin (Mfn) protein function. *J Cell Sci* 119: 4913–4925, 2006.
92. **Eura Y, Ishihara N, Yokota S, Mihara K.** Two mitofusin proteins, mammalian homologues of FZO, with distinct functions are both required for mitochondrial fusion. *J Biochem* 134: 333–344, 2003.
93. **Felber JP, Ferrannini E, Golay A, Meyer HU, Theibaud D, Curchod B, Maeder E, Jequier E, DeFronzo RA.** Role of lipid oxidation in pathogenesis of insulin resistance of obesity and type II diabetes. *Diabetes* 36: 1341–1350, 1987.
94. **Ferrari G, Lamantea E, Donati A, Filosto M, Briem E, Carrara F, Parini R, Simonati A, Santer R, Zeviani M.** Infantile hepatocerebral syndromes associated with mutations in the mitochondrial DNA polymerase-gammaA. *Brain* 128: 723–731, 2005.
95. **Ferre M, Amati-Bonneau P, Tourmen Y, Malthiery Y, Reynier P.** eOPA1: an online database for OPA1 mutations. *Hum Mutat* 25: 423–428, 2005.
96. **Fitts RH, Booth FW, Winder WW, Holloszy JO.** Skeletal muscle respiratory capacity, endurance, and glycogen utilization. *Am J Physiol* 228: 1029–1033, 1975.
97. **Frank S, Gaume B, Bergmann-Leitner ES, Leitner WW, Robert EG, Catez F, Smith CL, Youle RJ.** The role of dynamin-

related protein 1, a mediator of mitochondrial fission, in apoptosis. *Dev Cell* 1: 515–525, 2001.
98. **Fransson S, Ruusala A, Aspenstrom P.** The atypical Rho GTPases Miro-1 and Miro-2 have essential roles in mitochondrial trafficking. *Biochem Biophys Res Commun* 344: 500–510, 2006.
99. **Franzini-Armstrong C.** ER-mitochondria communication. How privileged? *Physiology* 22: 261–268, 2007.
100. **Frederick RL, McCaffery JM, Cunningham KW, Okamoto K, Shaw JM.** Yeast Miro GTPase, Gem1p, regulates mitochondrial morphology via a novel pathway. *J Cell Biol* 167: 87–98, 2004.
101. **Frezza C, Cipolat S, Martins dB, Micaroni M, Beznoussenko GV, Rudka T, Bartoli D, Polishuck RS, Danial NN, De Strooper B, Scorrano L.** OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion. *Cell* 126: 177–189, 2006.
102. **Frieden M, James D, Castelbou C, Danckaert A, Martinou JC, Demaurex N.** Ca^{2+} homeostasis during mitochondrial fragmentation and perinuclear clustering induced by hFis1. *J Biol Chem* 279: 22704–22714, 2004.
103. **Fukushima NH, Brisch E, Keegan BR, Bleazard W, Shaw JM.** The GTPase effector domain sequence of the Dnm1p GTPase regulates self-assembly and controls a rate-limiting step in mitochondrial fission. *Mol Biol Cell* 12: 2756–2766, 2001.
104. **Gallop JL, Butler PJ, McMahon HT.** Endophilin and CtBP/BARS are not acyl transferases in endocytosis or Golgi fission. *Nature* 438: 675–678, 2005.
105. **Gallop JL, Jao CC, Kent HM, Butler PJ, Evans PR, Langen R, McMahon HT.** Mechanism of endophilin N-BAR domain-mediated membrane curvature. *EMBO J* 25: 2898–2910, 2006.
106. **Gandre-Babbe S, van der Bliek AM.** The novel tail-anchored membrane protein Mff controls mitochondrial and peroxisomal fission in mammalian cells. *Mol Biol Cell* 19: 2402–2412, 2008.
107. **Garrido N, Griparic L, Jokitalo E, Wartiovaara J, van der Bliek AM, Spelbrink JN.** Composition and dynamics of human mitochondrial nucleoids. *Mol Biol Cell* 14: 1583–1596, 2003.
108. **Gastaldi G, Russell A, Golay A, Giacobino JP, Habicht F, Barthassat V, Muzzin P, Bobbioni-Harsch E.** Upregulation of peroxisome proliferator-activated receptor gamma coactivator gene (PGC1A) during weight loss is related to insulin sensitivity but not to energy expenditure. *Diabetologia* 50: 2348–2355, 2007.
109. **Germain M, Mathai JP, McBride HM, Shore GC.** Endoplasmic reticulum BIK initiates DRP1-regulated remodelling of mitochondrial cristae during apoptosis. *EMBO J* 24: 1546–1556, 2005.
110. **Gniuli D, Rosa G, Manco M, Scarfone A, Vega N, Greco AV, Castagneto M, Vidal H, Mingrone G.** Changes in fat mass influence SREBP-1c and UCP-2 gene expression in formerly obese subjects. *Obes Res* 13: 567–573, 2005.
111. **Gollnick PD, Armstrong RB, Saubert CW, Piehl K, Saltin B.** Enzyme activity and fiber composition in skeletal muscle of untrained and trained men. *J Appl Physiol* 33: 312–319, 1972.
112. **Gomes LC, Scorrano L.** High levels of Fis1, a pro-fission mitochondrial protein, trigger autophagy. *Biochim Biophys Acta* 1777: 860–866, 2008.
113. **Greco AV, Mingrone G, Giancaterini A, Manco M, Morroni M, Cinti S, Granzotto M, Vettor R, Camastra S, Ferrannini E.** Insulin resistance in morbid obesity: reversal with intramyocellular fat depletion. *Diabetes* 51: 144–151, 2002.
114. **Griffin EE, Graumann J, Chan DC.** The WD40 protein Caf4p is a component of the mitochondrial fission machinery and recruits Dnm1p to mitochondria. *J Cell Biol* 170: 237–248, 2005.
115. **Griparic L, Kanazawa T, van der Bliek AM.** Regulation of the mitochondrial dynamin-like protein Opa1 by proteolytic cleavage. *J Cell Biol* 178: 757–764, 2007.
116. **Griparic L, van der Wel NN, Orozco IJ, Peters PJ, 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.
117. **Guan K, Farh L, Marshall TK, Deschenes RJ.** Normal mitochondrial structure and genome maintenance in yeast requires the dynamin-like product of the MGM1 gene. *Curr Genet* 24: 141–148, 1993.
118. **Guidone C, Manco M, Valera-Mora E, Iaconelli A, Gniuli D, Mari A, Nanni G, Castagneto M, Calvani M, Mingrone G.** Mechanisms of recovery from type 2 diabetes after malabsorptive bariatric surgery. *Diabetes* 55: 2025–2031, 2006.
119. **Guillery O, Malka F, Landes T, Guillou E, Blackstone C, Lombes A, Belenguer P, Arnoult D, Rojo M.** Metalloprotease-mediated OPA1 processing is modulated by the mitochondrial membrane potential. *Biol Cell* 100: 315–325, 2008.
120. **Guo X, Macleod GT, Wellington A, Hu F, Panchumarthi S, Schoenfield M, Marin L, Charlton MP, Atwood HL, Zinsmaier KE.** The GTPase dMiro is required for axonal transport of mitochondria to *Drosophila* synapses. *Neuron* 47: 379–393, 2005.
121. **Guo YH, Chen K, Gao W, Li Q, Chen L, Wang GS, Tang J.** Overexpression of Mitofusin 2 inhibited oxidized low-density lipoprotein induced vascular smooth muscle cell proliferation and reduced atherosclerotic lesion formation in rabbit. *Biochem Biophys Res Commun* 363: 411–417, 2007.
122. **Hajek P, Chomyn A, Attardi G.** Identification of a novel mitochondrial complex containing mitofusin 2 and stomatin-like protein 2. *J Biol Chem* 282: 5670–5681, 2007.
123. **Hales KG, Fuller MT.** Developmentally regulated mitochondrial fusion mediated by a conserved, novel, and predicted GTPase. *Cell* 90: 121–129, 1997.
124. **Hamann A, Benecke H, Marchand-Brustel Y, Susulic VS, Lowell BB, Flier JS.** Characterization of insulin resistance and NIDDM in transgenic mice with reduced brown fat. *Diabetes* 44: 1266–1273, 1995.
125. **Han XJ, Lu YF, Li SA, Kaitsuka T, Sato Y, Tomizawa K, Nairn AC, Takei K, Matsui H, Matsushita M.** CaM kinase I alpha-induced phosphorylation of Drp1 regulates mitochondrial morphology. *J Cell Biol* 182: 573–585, 2008.
126. **Harder Z, Zunino R, McBride H.** Sumo1 conjugates mitochondrial substrates and participates in mitochondrial fission. *Curr Biol* 14: 340–345, 2004.
127. **Hayashi J, Takemitsu M, Goto Y, Nonaka I.** Human mitochondria and mitochondrial genome function as a single dynamic cellular unit. *J Cell Biol* 125: 43–50, 1994.
128. **Herlan M, Vogel F, Bornhovd C, Neupert W, Reichert AS.** 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, 2003.
129. **Hermann GJ, King EJ, Shaw JM.** The yeast gene, MDM20, is necessary for mitochondrial inheritance and organization of the actin cytoskeleton. *J Cell Biol* 137: 141–153, 1997.
130. **Hermann GJ, Thatcher JW, Mills JP, Hales KG, Fuller MT, Nunnari J, Shaw JM.** Mitochondrial fusion in yeast requires the transmembrane GTPase Fzo1p. *J Cell Biol* 143: 359–373, 1998.
131. **Holloszy JO.** Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. *J Biol Chem* 242: 2278–2282, 1967.
132. **Honda S, Aihara T, Hontani M, Okubo K, Hirose S.** Mutational analysis of action of mitochondrial fusion factor mitofusin-2. *J Cell Sci* 118: 3153–3161, 2005.
133. **Hoppins S, Lackner L, Nunnari J.** The machines that divide and fuse mitochondria. *Annu Rev Biochem* 76: 751–780, 2007.
134. **Hotamisligil GS.** The role of TNFalpha and TNF receptors in obesity and insulin resistance. *J Intern Med* 245: 621–625, 1999.
135. **Hotamisligil GS, Budavari A, Murray D, Spiegelman BM.** Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-alpha. *J Clin Invest* 94: 1543–1549, 1994.
136. **Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM.** IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science* 271: 665–668, 1996.
137. **Hotamisligil GS, Shargill NS, Spiegelman BM.** Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 259: 87–91, 1993.
138. **Hoyt CS.** Autosomal dominant optic atrophy. A spectrum of disability. *Ophthalmology* 87: 245–251, 1980.
139. **Huang P, Yu T, Yoon Y.** Mitochondrial clustering induced by overexpression of the mitochondrial fusion protein Mfn2 causes mitochondrial dysfunction and cell death. *Eur J Cell Biol* 86: 289–302, 2007.

140. **Hudson G, Amati-Bonneau P, Blakely EL, Stewart JD, He L, Schaefer AM, Griffiths PG, Ahlqvist K, Suomalainen A, Reynier P, McFarland R, Turnbull DM, Chinnery PF, Taylor RW.** Mutation of OPA1 causes dominant optic atrophy with external ophthalmoplegia, ataxia, deafness and multiple mitochondrial DNA deletions: a novel disorder of mtDNA maintenance. *Brain* 131: 329–337, 2008.
141. **Hwa JJ, Hiller MA, Fuller MT, Santel A.** Differential expression of the *Drosophila* mitofusin genes fuzzy onions (fzo) and dmfn. *Mech Dev* 116: 213–216, 2002.
142. **Imoto M, Tachibana I, Urrutia R.** Identification and functional characterization of a novel human protein highly related to the yeast dynamin-like GTPase Vps1p. *J Cell Sci* 111: 1341–1349, 1998.
143. **Ingerman E, Perkins EM, Marino M, Mears JA, McCaffery JM, Hinshaw JE, Nunnari J.** Dnm1 forms spirals that are structurally tailored to fit mitochondria. *J Cell Biol* 170: 1021–1027, 2005.
144. **Ishihara N, Eura Y, Mihara K.** Mitofusin 1 and 2 play distinct roles in mitochondrial fusion reactions via GTPase activity. *J Cell Sci* 117: 6535–6546, 2004.
145. **Ishihara N, Fujita Y, Oka T, Mihara K.** Regulation of mitochondrial morphology through proteolytic cleavage of OPA1. *EMBO J* 25: 2966–2977, 2006.
146. **Jacobs HT, Lehtinen SK, Spelbrink JN.** No sex please, we're mitochondria: a hypothesis on the somatic unit of inheritance of mammalian mtDNA. *Bioessays* 22: 564–572, 2000.
147. **Jagasia R, Grote P, Westermann B, Conradt B.** DRP-1-mediated mitochondrial fragmentation during EGL-1-induced cell death in *C. elegans*. *Nature* 433: 754–760, 2005.
148. **James DI, Parone PA, Mattenberger Y, Martinou JC.** hFis1, a novel component of the mammalian mitochondrial fission machinery. *J Biol Chem* 278: 36373–36379, 2003.
149. **Jeyaraju DV, Xu L, Letellier MC, Bandaru S, Zunino R, Berg EA, McBride HM, Pellegrini L.** Phosphorylation and cleavage of presenilin-associated rhomboid-like protein (PARL) promotes changes in mitochondrial morphology. *Proc Natl Acad Sci USA* 103: 18562–18567, 2006.
150. **Jiang GJ, Pan L, Huang XY, Han M, Wen JK, Sun FZ.** Expression of HSG is essential for mouse blastocyst formation. *Biochem Biophys Res Commun* 335: 351–355, 2005.
151. **Jofuku A, Ishihara N, Mihara K.** Analysis of functional domains of rat mitochondrial Fis1, the mitochondrial fission-stimulating protein. *Biochem Biophys Res Commun* 333: 650–659, 2005.
152. **Johnston PB, Gaster RN, Smith VC, Tripathi RC.** A clinicopathologic study of autosomal dominant optic atrophy. *Am J Ophthalmol* 88: 868–875, 1979.
153. **Johnston RL, Seller MJ, Behnam JT, Burdon MA, Spalton DJ.** Dominant optic atrophy. Refining the clinical diagnostic criteria in light of genetic linkage studies. *Ophthalmology* 106: 123–128, 1999.
154. **Jones BA, Fangman WL.** Mitochondrial DNA maintenance in yeast requires a protein containing a region related to the GTP-binding domain of dynamin. *Genes Dev* 6: 380–389, 1992.
155. **Kamimoto T, Nagai Y, Onogi H, Muro Y, Wakabayashi T, Hagiwara M.** Dymple, a novel dynamin-like high molecular weight GTPase lacking a proline-rich carboxyl-terminal domain in mammalian cells. *J Biol Chem* 273: 1044–1051, 1998.
156. **Kaplan ML, Oh SS.** Oxygen consumption of muscles from *ob/ob* and *Ay/a* strains of obese mice. *Int J Obes* 15: 809–812, 1991.
157. **Karbowski M, Arnoult D, Chen H, Chan DC, Smith CL, 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.
158. **Karbowski M, Jeong SY, Youle RJ.** Endophilin B1 is required for the maintenance of mitochondrial morphology. *J Cell Biol* 166: 1027–1039, 2004.
159. **Karbowski M, Lee YJ, Gaume B, Jeong SY, Frank S, Nechushtan A, Santel A, Fuller M, Smith CL, Youle RJ.** Spatial and temporal association of Bax with mitochondrial fission sites, Drp1, Mfn2 during apoptosis. *J Cell Biol* 159: 931–938, 2002.
160. **Karbowski M, Norris KL, Cleland MM, Jeong SY, Youle RJ.** Role of Bax and Bak in mitochondrial morphogenesis. *Nature* 443: 658–662, 2006.
161. **Kelley DE, He J, Menshikova EV, Ritov VB.** Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes* 51: 2944–2950, 2002.
162. **Kelley DE, Mandarino LJ.** Fuel selection in human skeletal muscle in insulin resistance: a reexamination. *Diabetes* 49: 677–683, 2000.
163. **Kemmer FW, Berger M, Herberg L, Gries FA, Wirdeier A, Becker K.** Glucose metabolism in perfused skeletal muscle. Demonstration of insulin resistance in the obese Zucker rat. *Biochem J* 178: 733–741, 1979.
164. **Kemper C, Habib SJ, Engl G, Heckmeyer P, Dimmer KS, Rapaport D.** Integration of tail-anchored proteins into the mitochondrial outer membrane does not require any known import components. *J Cell Sci* 121: 1990–1998, 2008.
165. **Kijima K, Numakura C, Izumino H, Umetsu K, Nezu A, Shiiki T, Ogawa M, Ishizaki Y, Kitamura T, Shozawa Y, Hayasaka K.** Mitochondrial GTPase mitofusin 2 mutation in Charcot-Marie-Tooth neuropathy type 2A. *Hum Genet* 116: 23–27, 2005.
166. **Kim HJ, Higashimori T, Park SY, Choi H, Dong J, Kim YJ, Noh HL, Cho YR, Cline G, Kim YB, Kim JK.** Differential effects of interleukin-6 and -10 on skeletal muscle and liver insulin action in vivo. *Diabetes* 53: 1060–1067, 2004.
167. **Kim JY, Hwang JM, Ko HS, Seong MW, Park BJ, Park SS.** Mitochondrial DNA content is decreased in autosomal dominant optic atrophy. *Neurology* 64: 966–972, 2005.
168. **Kimberg DV, Loeb JN.** Effects of cortisone administration on rat-liver mitochondria: support for concept of mitochondrial fusion. *J Cell Biol* 55: 635, 1972.
169. **Kjer B, Eiberg H, Kjer P, Rosenberg T.** Dominant optic atrophy mapped to chromosome 3q region. II. Clinical and epidemiological aspects. *Acta Ophthalmol Scand* 74: 3–7, 1996.
170. **Kjer P, Jensen OA, Klinken L.** Histopathology of eye, optic nerve and brain in a case of dominant optic atrophy. *Acta Ophthalmol* 61: 300–312, 1983.
171. **Klover PJ, Zimmers TA, Koniaris LG, Mooney RA.** Chronic exposure to interleukin-6 causes hepatic insulin resistance in mice. *Diabetes* 52: 2784–2789, 2003.
172. **Koch A, Thiemann M, Grabenbauer M, Yoon Y, McNiven MA, Schrader M.** Dynamin-like protein 1 is involved in peroxisomal fission. *J Biol Chem* 278: 8597–8605, 2003.
173. **Koch A, Yoon Y, Bonekamp NA, McNiven MA, Schrader M.** A role for Fis1 in both mitochondrial and peroxisomal fission in mammalian cells. *Mol Biol Cell* 16: 5077–5086, 2005.
174. **Korhonen JA, Gaspari M, Falkenberg M.** TWINKLE has 5′ → 3′ DNA helicase activity and is specifically stimulated by mitochondrial single-stranded DNA-binding protein. *J Biol Chem* 278: 48627–48632, 2003.
175. **Koshiba T, Detmer SA, Kaiser JT, Chen H, McCaffery JM, Chan DC.** Structural basis of mitochondrial tethering by mitofusin complexes. *Science* 305: 858–862, 2004.
176. **Kroemer G, Galluzzi L, Brenner C.** Mitochondrial membrane permeabilization in cell death. *Physiol Rev* 87: 99–163, 2007.
177. **Labrousse AM, Zappaterra MD, Rube DA, van der Bliek AM.** *C. elegans* dynamin-related protein DRP-1 controls severing of the mitochondrial outer membrane. *Mol Cell* 4: 815–826, 1999.
178. **Larson-Meyer DE, Heilbronn LK, Redman LM, Newcomer BR, Frisard MI, Anton S, Smith SR, Alfonso A, Ravussin E.** Effect of calorie restriction with or without exercise on insulin sensitivity, beta-cell function, fat cell size, and ectopic lipid in overweight subjects. *Diabetes Care* 29: 1337–1344, 2006.
179. **Lawson VH, Graham BV, Flanigan KM.** Clinical and electrophysiologic features of CMT2A with mutations in the mitofusin 2 gene. *Neurology* 65: 197–204, 2005.
180. **Lee S, Jeong SY, Lim WC, Kim S, Park YY, Sun X, Youle RJ, Cho H.** Mitochondrial fission and fusion mediators, hFis1 and OPA1, modulate cellular senescence. *J Biol Chem* 282: 22977–22983, 2007.
181. **Lee YJ, Jeong SY, Karbowski M, Smith CL, Youle RJ.** Roles of the mammalian mitochondrial fission and fusion mediators Fis1, Drp1, and Opa1 in apoptosis. *Mol Biol Cell* 15: 5001–5011, 2004.
182. **Legros F, Lombes A, Frachon P, 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.

183. **Legros F, Malka F, Frachon P, Lombes A, Rojo M.** Organization and dynamics of human mitochondrial DNA. *J Cell Sci* 117: 2653–2662, 2004.
184. **Lehninger AL.** *The Mitochondrion.* New York: Benjamin, 1965.
185. **Lehtinen SK, Hance N, El Meziane A, Juhola MK, Juhola KM, Karhu R, Spelbrink JN, Holt IJ, Jacobs HT.** Genotypic stability, segregation and selection in heteroplasmic human cell lines containing np 3243 mutant mtDNA. *Genetics* 154: 363–380, 2000.
186. **Lewis MR, Lewis WH.** Mitochondria in tissue culture. *Science* 39: 330–333, 1914.
187. **Li W, Bengtson MH, Ulbrich A, Matsuda A, Reddy VA, Orth A, Chanda SK, Batalov S, Joazeiro CA.** Genome-wide and functional annotation of human E3 ubiquitin ligases identifies MULAN, a mitochondrial E3 that regulates the organelle's dynamics and signaling. *PLoS ONE* 3: e1487, 2008.
188. **Li Z, Okamoto K, Hayashi Y, Sheng M.** The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. *Cell* 119: 873–887, 2004.
189. **Liesa M, Borda-d'Agua B, Medina-Gomez G, Lelliott CJ, Paz JC, Rojo M, Palacin M, Vidal-Puig A, Zorzano A.** Mitochondrial fusion is increased by the nuclear coactivator PGC-1beta. *PLoS ONE* 3: e3613, 2008.
190. **Lillioja S, Mott DM, Howard BV, Bennett PH, Yki-Jarvinen H, Freymond D, Nyomba BL, Zurlo F, Swinburn B, Bogardus C.** Impaired glucose tolerance as a disorder of insulin action. Longitudinal and cross-sectional studies in Pima Indians. *N Engl J Med* 318: 1217–1225, 1988.
191. **Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E, Knowler WC, Bennett PH, Bogardus C.** Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus. Prospective studies of Pima Indians. *N Engl J Med* 329: 1988–1992, 1993.
192. **Liu H, Nakagawa T, Kanematsu T, Uchida T, Tsuji S.** Isolation of 10 differentially expressed cDNAs in differentiated Neuro2a cells induced through controlled expression of the GD3 synthase gene. *J Neurochem* 72: 1781–1790, 1999.
193. **Loiseau D, Chevrollier A, Verny C, Guillet V, Gueguen N, Pou de Crescenzo MA, Ferre M, Malinge MC, Guichet A, Nicolas G, Amati-Bonneau P, Malthiery Y, Bonneau D, Reynier P.** Mitochondrial coupling defect in Charcot-Marie-Tooth type 2A disease. *Ann Neurol* 61: 315–323, 2007.
194. **Longley MJ, Clark S, Yu Wai MC, Hudson G, Durham SE, Taylor RW, Nightingale S, Turnbull DM, Copeland WC, Chinnery PF.** Mutant POLG2 disrupts DNA polymerase gamma subunits and causes progressive external ophthalmoplegia. *Am J Hum Genet* 78: 1026–1034, 2006.
195. **Magnusson J, Orth M, Lestienne P, Taanman JW.** Replication of mitochondrial DNA occurs throughout the mitochondria of cultured human cells. *Exp Cell Res* 289: 133–142, 2003.
196. **Malka F, Guillery O, Cifuentes-Diaz C, Guillou E, Belenguer P, Lombes A, Rojo M.** Separate fusion of outer and inner mitochondrial membranes. *EMBO Rep* 6: 853–859, 2005.
197. **Mannella CA.** Structure and dynamics of the mitochondrial inner membrane cristae. *Biochim Biophys Acta* 1763: 542–548, 2006.
198. **Mannella CA.** The relevance of mitochondrial membrane topology to mitochondrial function. *Biochim Biophys Acta* 1762: 140–147, 2006.
199. **Marchbank NJ, Craig JE, Leek JP, Toohey M, Churchill AJ, Markham AF, Mackey DA, Toomes C, Inglehearn CF.** Deletion of the OPA1 gene in a dominant optic atrophy family: evidence that haploinsufficiency is the cause of disease. *J Med Genet* 39: e47, 2002.
200. **Margineantu DH, Gregory CW, Sundell L, Sherwood SW, Beechem JM, Capaldi RA.** Cell cycle dependent morphology changes and associated mitochondrial DNA redistribution in mitochondria of human cell lines. *Mitochondrion* 1: 425–435, 2002.
201. **Martinou JC, Youle RJ.** Which came first, the cytochrome *c* release or the mitochondrial fission? *Cell Death Differ* 13: 1291–1295, 2006.
202. **Mayorov VI, Lowrey AJ, Biousse V, Newman NJ, Cline SD, Brown MD.** Mitochondrial oxidative phosphorylation in autosomal dominant optic atrophy. *BMC Biochem* 9: 22, 2008.
203. **McBride HM, Neuspiel M, Wasiak S.** Mitochondria: more than just a powerhouse. *Curr Biol* 16: R551–R560, 2006.
204. **McConnell SJ, Stewart LC, Talin A, Yaffe MP.** Temperature-sensitive yeast mutants defective in mitochondrial inheritance. *J Cell Biol* 111: 967–976, 1990.
205. **Meeusen S, McCaffery JM, Nunnari J.** Mitochondrial fusion intermediates revealed in vitro. *Science* 305: 1747–1752, 2004.
206. **Merkwirth C, Dargazanli S, Tatsuta T, Geimer S, Lower B, Wunderlich FT, Kleist-Retzow JC, Waisman A, Westermann B, Langer T.** Prohibitins control cell proliferation and apoptosis by regulating OPA1-dependent cristae morphogenesis in mitochondria. *Genes Dev* 22: 476–488, 2008.
207. **Messerschmitt M, Jakobs S, Vogel F, Fritz S, Dimmer KS, Neupert W, Westermann B.** The inner membrane protein Mdm33 controls mitochondrial morphology in yeast. *J Cell Biol* 160: 553–564, 2003.
208. **Mingrone G, Manco M, Calvani M, Castagneto M, Naon D, Zorzano A.** Could the low level of expression of the gene encoding skeletal muscle mitofusin-2 account for the metabolic inflexibility of obesity? *Diabetologia* 48: 2108–2114, 2005.
209. **Mingrone G, Rosa G, Greco AV, Manco M, Vega N, Hesselink MK, Castagneto M, Schrauwen P, Vidal H.** Decreased uncoupling protein expression and intramyocytic triglyceride depletion in formerly obese subjects. *Obes Res* 11: 632–640, 2003.
210. **Mingrone G, Rosa G, Greco AV, Manco M, Vega N, Nanni G, Castagneto M, Vidal H.** Intramyocitic lipid accumulation and SREBP-1c expression are related to insulin resistance and cardiovascular risk in morbid obesity. *Atherosclerosis* 170: 155–161, 2003.
211. **Mintz GS, Tinana A, Hong MK, Lee CW, Kim JJ, Fearnot NE, Park SW, Park SJ, Weissman NJ.** Impact of preinterventional arterial remodeling on neointimal hyperplasia after implantation of (non-polymer-encapsulated) paclitaxel-coated stents: a serial volumetric intravascular ultrasound analysis from the ASian Paclitaxel-Eluting Stent Clinical Trial (ASPECT). *Circulation* 108: 1295–1298, 2003.
212. **Miyakawa I, Aoi H, Sando N, Kuroiwa T.** Fluorescence microscopic studies of mitochondrial nucleoids during meiosis and sporulation in the yeast, *Saccharomyces cerevisiae*. *J Cell Sci* 66: 21–38, 1984.
213. **Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstrale M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, Spiegelman B, Lander ES, Hirschhorn JN, Altshuler D, Groop LC.** PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet* 34: 267–273, 2003.
214. **Nakamura N, Kimura Y, Tokuda M, Honda S, Hirose S.** MARCH-V is a novel mitofusin 2- and Drp1-binding protein able to change mitochondrial morphology. *EMBO Rep* 7: 1019–1022, 2006.
215. **Nelis E, Erdem S, Van Den Bergh PY, Belpaire-Dethiou MC, Ceuterick C, Van G, V, Cuesta A, Pedrola L, Palau F, Gabreels-Festen AA, Verellen C, Tan E, Demirci M, Van Broeckhoven C, De Jonghe P, Topaloglu H, Timmerman V.** Mutations in GDAP1: autosomal recessive CMT with demyelination and axonopathy. *Neurology* 59: 1865–1872, 2002.
216. **Neuspiel M, Zunino R, Gangaraju S, Rippstein P, McBride H.** Activated mitofusin 2 signals mitochondrial fusion, interferes with Bax activation, and reduces susceptibility to radical induced depolarization. *J Biol Chem* 280: 25060–25070, 2005.
217. **Neutzner A, Youle RJ.** Instability of the mitofusin Fzo1 regulates mitochondrial morphology during the mating response of the yeast *Saccharomyces cerevisiae*. *J Biol Chem* 280: 18598–18603, 2005.
218. **Niemann A, Ruegg M, La P, V, Schenone A, 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.
219. **Nisoli E, Tonello C, Cardile A, Cozzi V, Bracale R, Tedesco L, Falcone S, Valerio A, Cantoni O, Clementi E, Moncada S, Carruba MO.** Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. *Science* 310: 314–317, 2005.
220. **Nowikovsky K, Froschauer EM, Zsurka G, Samaj J, Reipert S, Kolisek M, Wiesenberger G, 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.

221. **Nunnari J, Marshall WF, Straight A, Murray A, Sedat JW, Walter P.** Mitochondrial transmission during mating in *Saccharomyces cerevisiae* is determined by mitochondrial fusion and fission and the intramitochondrial segregation of mitochondrial DNA. *Mol Biol Cell* 8: 1233–1242, 1997.
222. **Okamoto K, Shaw JM.** Mitochondrial morphology and dynamics in yeast and multicellular eukaryotes. *Annu Rev Genet* 39: 503–536, 2005.
223. **Olichon A, Baricault L, Gas N, Guillou E, Valette A, Belenguer P, 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.
224. **Olichon A, Elachouri G, Baricault L, Delettre C, Belenguer P, Lenaers G.** OPA1 alternate splicing uncouples an evolutionary conserved function in mitochondrial fusion from a vertebrate restricted function in apoptosis. *Cell Death Differ* 14: 682–692, 2007.
225. **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.** The human dynamin-related protein OPA1 is anchored to the mitochondrial inner membrane facing the inter-membrane space. *FEBS Lett* 523: 171–176, 2002.
226. **Olichon A, Guillou E, Delettre C, Landes T, Arnaune-Pelloquin L, Emorine LJ, Mils V, Daloyau M, Hamel C, Amati-Bonneau P, Bonneau D, Reynier P, Lenaers G, Belenguer P.** Mitochondrial dynamics and disease, OPA1. *Biochim Biophys Acta* 1763: 500–509, 2006.
227. **Olichon A, Landes T, Arnaune-Pelloquin L, Emorine LJ, Mils V, Guichet A, Delettre C, Hamel C, Amati-Bonneau P, Bonneau D, Reynier P, Lenaers G, Belenguer P.** Effects of OPA1 mutations on mitochondrial morphology and apoptosis: relevance to ADOA pathogenesis. *J Cell Physiol* 211: 423–430, 2007.
228. **Parone PA, Da Cruz S, Tondera D, Mattenberger Y, James DI, Maechler P, Barja F, Martinou JC.** Preventing mitochondrial fission impairs mitochondrial function and leads to loss of mitochondrial DNA. *PLoS ONE* 3: e3257, 2008.
229. **Parone PA, James DI, Da Cruz S, Mattenberger Y, Donze O, Barja F, Martinou JC.** Inhibiting the mitochondrial fission machinery does not prevent Bax/Bak-dependent apoptosis. *Mol Cell Biol* 26: 7397–7408, 2006.
230. **Patti ME, Butte AJ, Crunkhorn S, Cusi K, Berria R, Kashyap S, Miyazaki Y, Kohane I, Costello M, Saccone R, Landaker EJ, Goldfine AB, Mun E, DeFronzo R, Finlayson J, Kahn CR, Mandarino LJ.** Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1. *Proc Natl Acad Sci USA* 100: 8466–8471, 2003.
231. **Pedrola L, Espert A, Valdes-Sanchez T, Sanchez-Piris M, Sirkowski EE, Scherer SS, Farinas I, Palau F.** Cell expression of GDAP1 in the nervous system and pathogenesis of Charcot-Marie-Tooth type 4A disease. *J Cell Mol Med* 12: 679–689, 2008.
232. **Pedrola L, Espert A, Wu X, Claramunt R, Shy ME, 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.
233. **Pesch UE, Leo-Kottler B, Mayer S, Jurklies B, Kellner U, Apfelstedt-Sylla E, Zrenner E, Alexander C, Wissinger B.** OPA1 mutations in patients with autosomal dominant optic atrophy and evidence for semi-dominant inheritance. *Hum Mol Genet* 10: 1359–1368, 2001.
234. **Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, DiPietro L, Cline GW, Shulman GI.** Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science* 300: 1140–1142, 2003.
235. **Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI.** Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med* 350: 664–671, 2004.
236. **Pich S, Bach D, Briones P, Liesa M, Camps M, Testar X, Palacin M, 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.
237. **Pickup JC, Chusney GD, Thomas SM, Burt D.** Plasma interleukin-6, tumour necrosis factor alpha and blood cytokine production in type 2 diabetes. *Life Sci* 67: 291–300, 2000.
238. **Pilegaard H, Saltin B, Neufer PD.** Exercise induces transient transcriptional activation of the PGC-1alpha gene in human skeletal muscle. *J Physiol* 546: 851–858, 2003.
239. **Pitts KR, McNiven MA, Yoon Y.** Mitochondria-specific function of the dynamin family protein DLP1 is mediated by its C-terminal domains. *J Biol Chem* 279: 50286–50294, 2004.
240. **Pitts KR, Yoon Y, Krueger EW, 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.
241. **Poole AC, Thomas RE, Andrews LA, McBride HM, Whitworth AJ, Pallanck LJ.** The PINK1/Parkin pathway regulates mitochondrial morphology. *Proc Natl Acad Sci USA* 105: 1638–1643, 2008.
242. **Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM.** C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* 286: 327–334, 2001.
243. **Praefcke GJ, McMahon HT.** The dynamin superfamily: universal membrane tubulation and fission molecules? *Nat Rev Mol Cell Biol* 5: 133–147, 2004.
244. **Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM.** A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* 92: 829–839, 1998.
245. **Ramachandran R, Surka M, Chappie JS, Fowler DM, Foss TR, Song BD, Schmid SL.** The dynamin middle domain is critical for tetramerization and higher-order self-assembly. *EMBO J* 26: 559–566, 2007.
246. **Rapaport D.** Finding the right organelle. Targeting signals in mitochondrial outer-membrane proteins. *EMBO Rep* 4: 948–952, 2003.
247. **Rapaport D, Brunner M, Neupert W, Westermann B.** Fzo1p is a mitochondrial outer membrane protein essential for the biogenesis of functional mitochondria in *Saccharomyces cerevisiae*. *J Biol Chem* 273: 20150–20155, 1998.
248. **Rauch A, Schellmoser S, Kraus C, Dorr HG, Trautmann U, Altherr MR, Pfeiffer RA, Reis A.** First known microdeletion within the Wolf-Hirschhorn syndrome critical region refines genotype-phenotype correlation. *Am J Med Genet* 99: 338–342, 2001.
249. **Reichert AS, Neupert W.** Contact sites between the outer and inner membrane of mitochondria-role in protein transport. *Biochim Biophys Acta* 1592: 41–49, 2002.
250. **Rieusset J, Bouzakri K, Chevillotte E, Ricard N, Jacquet D, Bastard JP, Laville M, Vidal H.** Suppressor of cytokine signaling 3 expression and insulin resistance in skeletal muscle of obese and type 2 diabetic patients. *Diabetes* 53: 2232–2241, 2004.
251. **Rizzuto R, Pinton P, Carrington W, Fay FS, Fogarty KE, Lifshitz LM, Tuft RA, Pozzan T.** Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca^{2+} responses. *Science* 280: 1763–1766, 1998.
252. **Rojo M, Legros F, Chateau D, Lombes A.** Membrane topology and mitochondrial targeting of mitofusins, ubiquitous mammalian homologs of the transmembrane GTPase Fzo. *J Cell Sci* 115: 1663–1674, 2002.
253. **Sandre-Giovannoli A, Chaouch M, Boccaccio I, Bernard R, Delague V, Grid D, Vallat JM, Levy N, Megarbane A.** Phenotypic and genetic exploration of severe demyelinating and secondary axonal neuropathies resulting from GDAP1 nonsense and splicing mutations. *J Med Genet* 40: e87, 2003.
254. **Santel A, Frank S.** Shaping mitochondria: the complex posttranslational regulation of the mitochondrial fission protein DRP1. *IUBMB Life* 60: 448–455, 2008.
255. **Santel A, Frank S, Gaume B, Herrler M, Youle RJ, Fuller MT.** Mitofusin-1 protein is a generally expressed mediator of mitochondrial fusion in mammalian cells. *J Cell Sci* 116: 2763–2774, 2003.
256. **Santel A, Fuller MT.** Control of mitochondrial morphology by a human mitofusin. *J Cell Sci* 114: 867–874, 2001.
257. **Saotome M, Safiulina D, Szabadkai G, Das S, Fransson A, Aspenstrom P, Rizzuto R, Hajnoczky G.** Bidirectional Ca^{2+}-dependent control of mitochondrial dynamics by the Miro GTPase. *Proc Natl Acad Sci USA* 105: 20728–20733, 2008.
258. **Sarzi E, Goffart S, Serre V, Chretien D, Slama A, Munnich A, Spelbrink JN, Rotig A.** Twinkle helicase (PEO1) gene mutation

causes mitochondrial DNA depletion. *Ann Neurol* 62: 579–587, 2007.
259. **Satoh M, Hamamoto T, Seo N, Kagawa Y, Endo H.** Differential sublocalization of the dynamin-related protein OPA1 isoforms in mitochondria. *Biochem Biophys Res Commun* 300: 482–493, 2003.
260. **Scorrano L, Ashiya M, Buttle K, Weiler S, Oakes SA, Mannella CA, Korsmeyer SJ.** A distinct pathway remodels mitochondrial cristae and mobilizes cytochrome *c* during apoptosis. *Dev Cell* 2: 55–67, 2002.
261. **Senderek J, Bergmann C, Ramaekers VT, Nelis E, Bernert G, Makowski A, Zuchner S, De Jonghe P, Rudnik-Schoneborn S, Zerres K, Schroder JM.** Mutations in the ganglioside-induced differentiation-associated protein-1 (GDAP1) gene in intermediate type autosomal recessive Charcot-Marie-Tooth neuropathy. *Brain* 126: 642–649, 2003.
262. **Senn JJ, Klover PJ, Nowak IA, Zimmers TA, Koniaris LG, Furlanetto RW, Mooney RA.** Suppressor of cytokine signaling-3 (SOCS-3), a potential mediator of interleukin-6-dependent insulin resistance in hepatocytes. *J Biol Chem* 278: 13740–13746, 2003.
263. **Sesaki H, Jensen RE.** Ugo1p links the Fzo1p and Mgm1p GTPases for mitochondrial fusion. *J Biol Chem* 279: 28298–28303, 2004.
264. **Seth RB, Sun L, Ea CK, Chen ZJ.** Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. *Cell* 122: 669–682, 2005.
265. **Sevilla T, Cuesta A, Chumillas MJ, Mayordomo F, Pedrola L, Palau F, Vilchez JJ.** Clinical, electrophysiological and morphological findings of Charcot-Marie-Tooth neuropathy with vocal cord palsy and mutations in the GDAP1 gene. *Brain* 126: 2023–2033, 2003.
266. **Shaw JM, Nunnari J.** Mitochondrial dynamics and division in budding yeast. *Trends Cell Biol* 12: 178–184, 2002.
267. **Shepard KA, Yaffe MP.** The yeast dynamin-like protein, Mgm1p, functions on the mitochondrial outer membrane to mediate mitochondrial inheritance. *J Cell Biol* 144: 711–720, 1999.
268. **Sheridan C, Delivani P, Cullen SP, Martin SJ.** Bax- or Bak-induced mitochondrial fission can be uncoupled from cytochrome *c* release. *Mol Cell* 31: 570–585, 2008.
269. **Shin HW, Shinotsuka C, Torii S, Murakami K, Nakayama K.** Identification and subcellular localization of a novel mammalian dynamin-related protein homologous to yeast Vps1p and Dnm1p. *J Biochem* 122: 525–530, 1997.
270. **Simoneau JA, Kelley DE.** Altered glycolytic and oxidative capacities of skeletal muscle contribute to insulin resistance in NIDDM. *J Appl Physiol* 83: 166–171, 1997.
271. **Skre H.** Genetic and clinical aspects of Charcot-Marie-Tooth's disease. *Clin Genet* 6: 98–118, 1974.
272. **Skulachev VP.** Mitochondrial filaments and clusters as intracellular power-transmitting cables. *Trends Biochem Sci* 26: 23–29, 2001.
273. **Smirnova E, Griparic L, Shurland DL, van der Bliek AM.** Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells. *Mol Biol Cell* 12: 2245–2256, 2001.
274. **Smirnova E, Shurland DL, Ryazantsev SN, van der Bliek AM.** A human dynamin-related protein controls the distribution of mitochondria. *J Cell Biol* 143: 351–358, 1998.
275. **Smith DM.** The ontogenetic history of the mitochondria of the hepatic cell of the white rat. *J Morphol* 52: 485–511, 1931.
276. **Sogo LF, Yaffe MP.** Regulation of mitochondrial morphology and inheritance by Mdm10p, a protein of the mitochondrial outer membrane. *J Cell Biol* 126: 1361–1373, 1994.
277. **Song Z, Chen H, Fiket M, Alexander C, Chan DC.** OPA1 processing controls mitochondrial fusion and is regulated by mRNA splicing, membrane potential, Yme1L. *J Cell Biol* 178: 749–755, 2007.
278. **Soriano FX, Liesa M, Bach D, Chan DC, Palacin M, Zorzano A.** Evidence for a mitochondrial regulatory pathway defined by peroxisome proliferator-activated receptor-gamma coactivator-1 alpha, estrogen-related receptor-alpha, mitofusin 2. *Diabetes* 55: 1783–1791, 2006.
279. **Spelbrink JN, Li FY, Tiranti V, Nikali K, Yuan QP, Tariq M, Wanrooij S, Garrido N, Comi G, Morandi L, Santoro L, Toscano A, Fabrizi GM, Somer H, Croxen R, Beeson D, Poulton J, Suomalainen A, Jacobs HT, Zeviani M, Larsson C.** Human mitochondrial DNA deletions associated with mutations in the gene encoding Twinkle, a phage T7 gene 4-like protein localized in mitochondria. *Nat Genet* 28: 223–231, 2001.
280. **Spinazzi M, Cazzola S, Bortolozzi M, Baracca A, Loro E, Casarin A, Solaini G, Sgarbi G, Casalena G, Cenacchi G, Malena A, Frezza C, Carrara F, Angelini C, Scorrano L, Salviati L, Vergani L.** A novel deletion in the GTPase domain of OPA1 causes defects in mitochondrial morphology and distribution, but not in function. *Hum Mol Genet* 17: 3291–3302, 2008.
281. **Sreekumar R, Halvatsiotis P, Schimke JC, Nair KS.** Gene expression profile in skeletal muscle of type 2 diabetes and the effect of insulin treatment. *Diabetes* 51: 1913–1920, 2002.
282. **Stevens BJ.** Variation in number and volume of mitochondria in yeast according to growth-conditions: study based on serial sectioning and computer graphics reconstitution. *Biologie Cellulaire* 28: 37–56, 1977.
283. **Stevens BJ.** Mitochondrial structure. In: *The Molecular Biology of the Yeast Saccharomyces: Life Cycle snd Inheritance*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1981, vol. 1, p. 471–504.
284. **Stith RD, Luo J.** Endocrine and carbohydrate responses to interleukin-6 in vivo. *Circ Shock* 44: 210–215, 1994.
285. **Stojanovski D, Koutsopoulos OS, Okamoto K, Ryan MT.** Levels of human Fis1 at the mitochondrial outer membrane regulate mitochondrial morphology. *J Cell Sci* 117: 1201–1210, 2004.
286. **Storlien L, Oakes ND, Kelley DE.** Metabolic flexibility. *Proc Nutr Soc* 63: 363–368, 2004.
287. **Suter U, Scherer SS.** Disease mechanisms in inherited neuropathies. *Nat Rev Neurosci* 4: 714–726, 2003.
288. **Suzuki M, Jeong SY, Karbowski M, Youle RJ, Tjandra N.** The solution structure of human mitochondria fission protein Fis1 reveals a novel TPR-like helix bundle. *J Mol Biol* 334: 445–458, 2003.
289. **Szabadkai G, Simoni AM, Chami M, Wieckowski MR, Youle RJ, Rizzuto R.** Drp-1-dependent division of the mitochondrial network blocks intraorganellar Ca^{2+} waves and protects against Ca^{2+}-mediated apoptosis. *Mol Cell* 16: 59–68, 2004.
290. **Taguchi N, Ishihara N, Jofuku A, Oka T, Mihara K.** Mitotic phosphorylation of dynamin-related GTPase Drp1 participates in mitochondrial fission. *J Biol Chem* 282: 11521–11529, 2007.
291. **Takahashi Y, Karbowski M, Yamaguchi H, Kazi A, Wu J, Sebti SM, Youle RJ, Wang HG.** Loss of Bif-1 suppresses Bax/Bak conformational change and mitochondrial apoptosis. *Mol Cell Biol* 25: 9369–9382, 2005.
292. **Takamatsu C, Umeda S, Ohsato T, Ohno T, Abe Y, Fukuoh A, Shinagawa H, Hamasaki N, Kang D.** Regulation of mitochondrial D-loops by transcription factor A and single-stranded DNA-binding protein. *EMBO Rep* 3: 451–456, 2002.
293. **Tamai S, Iida H, Yokota S, Sayano T, Kiguchiya S, Ishihara N, Hayashi J, Mihara K, Oka T.** Characterization of the mitochondrial protein LETM1, which maintains the mitochondrial tubular shapes and interacts with the AAA-ATPase BCS1L. *J Cell Sci* 121: 2588–2600, 2008.
294. **Thomas DY, Wilkie D.** Recombination of mitochondrial drug-resistance factor in *Saccharomyces cerevisiae*. *Biochem Biophys Res Commun* 30: 368, 1968.
295. **Tieu Q, Okreglak V, Naylor K, Nunnari J.** The WD repeat protein, Mdv1p, functions as a molecular adaptor by interacting with Dnm1p and Fis1p during mitochondrial fission. *J Cell Biol* 158: 445–452, 2002.
296. **Tinel H, Cancela JM, Mogami H, Gerasimenko JV, Gerasimenko OV, Tepikin AV, Petersen OH.** Active mitochondria surrounding the pancreatic acinar granule region prevent spreading of inositol trisphosphate-evoked local cytosolic Ca^{2+} signals. *EMBO J* 18: 4999–5008, 1999.
297. **Toledo FG, Watkins S, Kelley DE.** Changes induced by physical activity and weight loss in the morphology of intermyofibrillar mitochondria in obese men and women. *J Clin Endocrinol Metab* 91: 3224–3227, 2006.
298. **Tondera D, Czauderna F, Paulick K, Schwarzer R, Kaufmann J, Santel A.** The mitochondrial protein MTP18 contributes to mitochondrial fission in mammalian cells. *J Cell Sci* 118: 3049–3059, 2005.
299. **Tondera D, Santel A, Schwarzer R, Dames S, Giese K, Klippel A, Kaufmann J.** Knockdown of MTP18, a novel phosphatidylino-

sitol 3-kinase-dependent protein, affects mitochondrial morphology and induces apoptosis. *J Biol Chem* 279: 31544–31555, 2004.
300. **Tsigos C, Papanicolaou DA, Kyrou I, Defensor R, Mitsiadis CS, Chrousos GP.** Dose-dependent effects of recombinant human interleukin-6 on glucose regulation. *J Clin Endocrinol Metab* 82: 4167–4170, 1997.
301. **Turcotte LP, Swenberger JR, Zavitz TM, Yee AJ.** Increased fatty acid uptake and altered fatty acid metabolism in insulin-resistant muscle of obese Zucker rats. *Diabetes* 50: 1389–1396, 2001.
302. **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.** Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *EMBO J* 27: 433–446, 2008.
303. **Twig G, Graf SA, Wikstrom JD, Mohamed H, Haigh SE, Elorza A, Deutsch M, Zurgil N, Reynolds N, Shirihai OS.** Tagging and tracking individual networks within a complex mitochondrial web with photoactivatable GFP. *Am J Physiol Cell Physiol* 291: C176–C184, 2006.
304. **Van der Bliek AM, Redelmeier TE, Damke H, Tisdale EJ, Meyerowitz EM, Schmid SL.** Mutations in human dynamin block an intermediate stage in coated vesicle formation. *J Cell Biol* 122: 553–563, 1993.
305. **Van Goethem G, Dermaut B, Lofgren A, Martin JJ, Van Broeckhoven C.** Mutation of POLG is associated with progressive external ophthalmoplegia characterized by mtDNA deletions. *Nat Genet* 28: 211–212, 2001.
306. **Varadi A, Johnson-Cadwell LI, Cirulli V, Yoon Y, Allan VJ, Rutter GA.** Cytoplasmic dynein regulates the subcellular distribution of mitochondria by controlling the recruitment of the fission factor dynamin-related protein-1. *J Cell Sci* 117: 4389–4400, 2004.
307. **Verhoeven K, Claeys KG, Zuchner S, Schroder JM, Weis J, Ceuterick C, Jordanova A, Nelis E, De Vriendt E, Van Hul M, Seeman P, Mazanec R, Saifi GM, Szigeti K, Mancias P, Butler IJ, Kochanski A, Ryniewicz B, De Bleecker J, Van den BP, Verellen C, Van Coster R, Goemans N, Auer-Grumbach M, Robberecht W, Milic R, V, Nevo Y, Tournev I, Guergueltcheva V, Roelens F, Vieregge P, Vinci P, Moreno MT, Christen HJ, Shy ME, Lupski JR, Vance JM, De Jonghe P, Timmerman V.** MFN2 mutation distribution and genotype/phenotype correlation in Charcot-Marie-Tooth type 2. *Brain* 129: 2093–2102, 2006.
308. **Vettor R, Mingrone G, Manco M, Granzotto M, Milan G, Scarda A, Lombardi A, Greco AV, Federspil G.** Reduced expression of uncoupling proteins-2 and -3 in adipose tissue in post-obese patients submitted to biliopancreatic diversion. *Eur J Endocrinol* 148: 543–550, 2003.
309. **Votruba M, Fitzke FW, Holder GE, Carter A, Bhattacharya SS, Moore AT.** Clinical features in affected individuals from 21 pedigrees with dominant optic atrophy. *Arch Ophthalmol* 116: 351–358, 1998.
310. **Votruba M, Moore AT, Bhattacharya SS.** Clinical features, molecular genetics, and pathophysiology of dominant optic atrophy. *J Med Genet* 35: 793–800, 1998.
311. **Wakabayashi T, Asano M, Kurono C.** Mechanism of the formation of megamitochondria induced by copper-chelating agents. I. On the formation process of megamitochondria in cuprizone-treated mouse liver. *Acta Pathol Jpn* 25: 15–37, 1975.
312. **Wakabayashi T, Green DE.** Membrane fusion in mitochondria. I. Ultrastructural basis for fusion. *J Electron Microsc* 26: 305–320, 1977.
313. **Walder K, Kerr-Bayles L, Civitarese A, Jowett J, Curran J, Elliott K, Trevaskis J, Bishara N, Zimmet P, Mandarino L, Ravussin E, Blangero J, Kissebah A, Collier GR.** The mitochondrial rhomboid protease PSARL is a new candidate gene for type 2 diabetes. *Diabetologia* 48: 459–468, 2005.
314. **Wang X, Su B, Fujioka H, Zhu X.** Dynamin-like protein 1 reduction underlies mitochondrial morphology and distribution abnormalities in fibroblasts from sporadic Alzheimer's disease patients. *Am J Pathol* 2008.
315. **Warram JH, Martin BC, Krolewski AS, Soeldner JS, Kahn CR.** Slow glucose removal rate and hyperinsulinemia precede the development of type II diabetes in the offspring of diabetic parents. *Ann Intern Med* 113: 909–915, 1990.
316. **Wasiak S, Zunino R, McBride HM.** Bax/Bak promote sumoylation of DRP1 and its stable association with mitochondria during apoptotic cell death. *J Cell Biol* 177: 439–450, 2007.
317. **Waterham HR, Koster J, van Roermund CW, Mooyer PA, Wanders RJ, Leonard JV.** A lethal defect of mitochondrial and peroxisomal fission. *N Engl J Med* 356: 1736–1741, 2007.
318. **Westermann B, Prokisch H.** Mitochondrial dynamics in filamentous fungi. *Fungal Genet Biol* 36: 91–97, 2002.
319. **Wong ED, Wagner JA, Scott SV, Okreglak V, Holewinske TJ, Cassidy-Stone A, Nunnari J.** The intramitochondrial dynamin-related GTPase, Mgm1p, is a component of a protein complex that mediates mitochondrial fusion. *J Cell Biol* 160: 303–311, 2003.
320. **Wright TJ, Ricke DO, Denison K, Abmayr S, Cotter PD, Hirschhorn K, Keinanen M, McDonald-McGinn D, Somer M, Spinner N, Yang-Feng T, Zackai E, Altherr MR.** A transcript map of the newly defined 165 kb Wolf-Hirschhorn syndrome critical region. *Hum Mol Genet* 6: 317–324, 1997.
321. **Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, Spiegelman BM.** Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* 98: 115–124, 1999.
322. **Yaffe MP.** The machinery of mitochondrial inheritance and behavior. *Science* 283: 1493–1497, 1999.
323. **Yamaguchi R, Lartigue L, Perkins G, Scott RT, Dixit A, Kushnareva Y, Kuwana T, Ellisman MH, Newmeyer DD.** Opa1-mediated cristae opening is Bax/Bak and BH3 dependent, required for apoptosis, and independent of Bak oligomerization. *Mol Cell* 31: 557–569, 2008.
324. **Yang Y, Ouyang Y, Yang L, Beal MF, McQuibban A, Vogel H, Lu B.** Pink1 regulates mitochondrial dynamics through interaction with the fission/fusion machinery. *Proc Natl Acad Sci USA* 105: 7070–7075, 2008.
325. **Yoon Y, Krueger EW, Oswald BJ, 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.
326. **Yoon Y, Pitts KR, Dahan S, McNiven MA.** A novel dynamin-like protein associates with cytoplasmic vesicles and tubules of the endoplasmic reticulum in mammalian cells. *J Cell Biol* 140: 779–793, 1998.
327. **Yoon Y, Pitts KR, McNiven MA.** Mammalian dynamin-like protein DLP1 tubulates membranes. *Mol Biol Cell* 12: 2894–2905, 2001.
328. **Yoon YS, Yoon DS, Lim IK, Yoon SH, Chung HY, Rojo M, Malka F, Jou MJ, Martinou JC, Yoon G.** Formation of elongated giant mitochondria in DFO-induced cellular senescence: involvement of enhanced fusion process through modulation of Fis1. *J Cell Physiol* 209: 468–480, 2006.
329. **Yu TZ, Robotham JL, Yoon Y.** Increased production of reactive oxygen species in hyperglycemic conditions requires dynamic change of mitochondrial morphology. *Proc Natl Acad Sci USA* 103: 2653–2658, 2006.
330. **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.** OPA1 mutations associated with dominant optic atrophy impair oxidative phosphorylation and mitochondrial fusion. *Brain* 131: 352–367, 2008.
331. **Zhang Y, Chan DC.** Structural basis for recruitment of mitochondrial fission complexes by Fis1. *Proc Natl Acad Sci USA* 104: 18526–18530, 2007.
332. **Zhu D, Kennerson ML, Walizada G, Zuchner S, Vance JM, Nicholson GA.** Charcot-Marie-Tooth with pyramidal signs is genetically heterogeneous: families with and without MFN2 mutations. *Neurology* 65: 496–497, 2005.
333. **Zhu PP, Patterson A, Stadler J, Seeburg DP, Sheng M, Blackstone C.** Intra- and intermolecular domain interactions of the C-terminal GTPase effector domain of the multimeric dynamin-like GTPase Drp1. *J Biol Chem* 279: 35967–35974, 2004.
334. **Zick M, Rabl R, Reichert AS.** Cristae formation-linking ultrastructure and function of mitochondria. *Biochim Biophys Acta* 1793: 5–19, 2009.

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

336. **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, De Jonghe P, Takahashi Y, Tsuji S, Pericak-Vance MA, Quattrone A, Battologlu E, Polyakov AV, Timmerman V, Schroder JM, Vance JM.** Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. *Nature Genet* 36: 449–451, 2004.

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