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Emerging functions of mammalian mitochondrial fusion and fission

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Mitochondria provide a myriad of services to the cell, including energy production, calcium buffering and regulation of apoptosis. How these diverse functions are coordinated among the hundreds of mitochondria in a given cell is largely unknown, but is probably dependent on the dynamic nature of mitochondria. In this review, we explore the latest developments in mitochondrial dynamics in mammals. These studies indicate that mitofusins and OPA1 are essential for mitochondrial fusion, whereas Fis1 and Drp1 are essential for mitochondrial fission. The overall morphology of the mitochondrial population depends on the relative activities of these two sets of proteins. In addition to the regulation of mitochondrial shape, these molecules also play important roles in cell and tissue physiology. Perturbation of mitochondrial fusion results in defects in mitochondrial membrane potential and respiration, poor cell growth and increased susceptibility to cell death. These cellular observations may explain why mitochondrial fusion is essential for embryonic development. Two inherited neuropathies, Charcot–Marie–Tooth type 2A and autosomal dominant optic atrophy, are caused by mutations in mitofusin 2 and OPA1, suggesting that proper regulation of mitochondrial dynamics is particularly vital to neurons. Mitochondrial fission accompanies several types of apoptotic cell death and appears important for progression of the apoptotic pathway. These studies provide insight into how mitochondria communicate with one another to coordinate mitochondrial function and morphology.

Recent work has highlighted the importance of mitochondrial dynamics in cell and animal physiology. Because mitochondria constantly fuse and divide, an imbalance of these two processes dramatically alters overall mitochondrial morphology (Fig. 1). Disruption of fusion causes the normal, tubular network of mitochondria to fragment into short rods or spheres (1–4). Conversely, disruption of fission generates elongated, interconnected tubules that often cluster perinuclearly (5–7). In addition to the regulation of mitochondrial morphology, it is now clear that mitochondrial dynamics plays additional roles in mitochondrial function.

FUSION AND FISSION PROTEINS

Mitofusins 1 and 2 (Mfn1, Mfn2) and OPA1 are essential for mitochondrial fusion (1,2,8–10) (Fig. 2A). These proteins are large GTPases localized to mitochondria. Mfn1 and Mfn2 show 81% homology and similar topologies (2,11–14). Both reside on the outer membrane with the N-terminal GTPase and a predicted coiled coil protruding into the cytosol (12). A transmembrane region then forms a U-shaped membrane anchor, ending in a cytosolic, C-terminal coiled coil.

OPA1 also possesses two predicted coiled coils, one just N-terminal to the GTPase and the other at the extreme C-terminal. OPA1 is an intermembrane space protein, closely associated with the inner membrane (15). However, there are at least eight splicing isoforms (16), some of which demonstrate different localizations (17). The yeast orthologue, Mgm1p, is produced as an integral inner membrane protein that is cleaved into a soluble intermembrane space protein (18–20). Both forms are required for Mgm1p function (18,21). Such post-translational modification has yet to be demonstrated in mammalian cells, but the mammalian PARL protease can substitute in Mgm1p cleavage (19).

Presumably, the Mfns and OPA1 work together to promote mitochondrial fusion. Consistent with this idea, OPA1 has been shown to tubulate mitochondria in an Mfn1-dependent manner (8). Mfn1 and Mfn2 can homodimerize or heterodimerize (2). However, no biochemical interactions have been shown between the Mfns and OPA1 in mammalian cells. In

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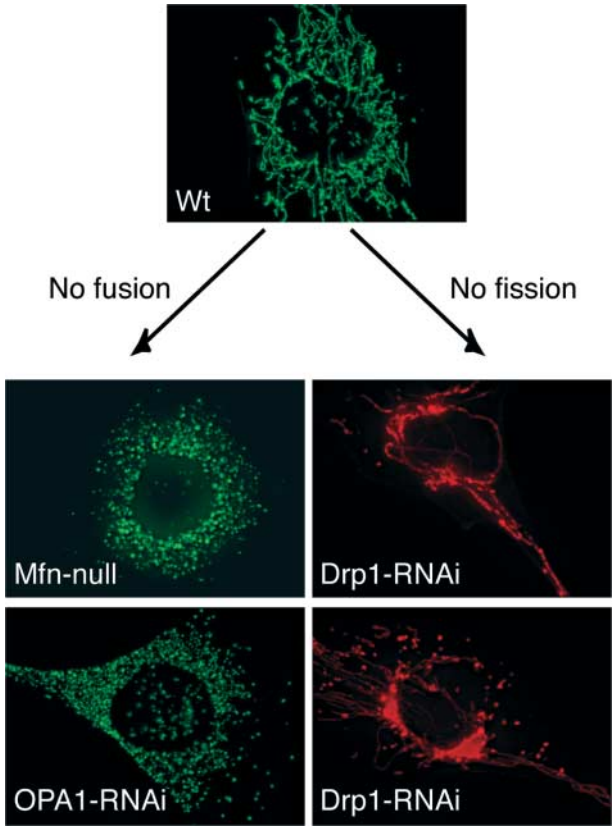

Figure 1. Relative rates of fusion and fission control mitochondrial morphology. Wild-type (Wt) mouse fibroblasts have tubular mitochondria. Inhibition of fusion (left panels) by deletion of both Mfn1 and Mfn2 (Mfn-null) or knock-down of OPA1 (OPA1–RNAi) causes mitochondrial fragmentation because of unopposed fission. Conversely, inhibition of fission (right panels) by knock-down of Drp1 (Drp–RNAi) causes excessively elongated and interconnected mitochondria that often collapse into perinuclear aggregates (bottom right).

yeast, immunoprecipitation studies have demonstrated weak but reproducible associations between Fzo1p (Mfn orthologue) and Mgm1p (20,22). In addition, another protein required for fusion, Ugo1p, interacts with this complex, but no mammalian homologue has been found.

On the fission side, at least two proteins, Fis1 and dynamin-related protein 1 (Drp1), are required in mammals (Fig. 2B). Drp1 exists largely in a cytosolic pool, but a fraction localizes to puncta on mitochondria (5). Drp1 contains a dynamin-like central domain and C-terminal GTPase effector domain (GED) in addition to its N-terminal GTPase. Intramolecular interactions between the GTPase and GED regions appear to be required for full GTPase and fission activities (23). Intermolecular oligomerization may also be necessary, as it is thought to be the case for dynamin. Given these similarities with dynamin, Drp1 has been proposed to couple GTP hydrolysis with mitochondrial membrane constriction and fission (5).

How is Drp1 recruited to mitochondria to mediate fission? One likely possibility is Fis1, a single-pass transmembrane protein that is anchored to the mitochondrial outer membrane by its C-terminal region (6). However, reduction of Fis1 levels by RNA interference (RNAi) does not disrupt Drp1 localization to mitochondria (24). A weak, direct interaction between Fis1 and Drp1 has been observed with recombinant proteins, but this association remains to be demonstrated *in vivo* (6). In contrast, yeast *fis1* mutants lose most Dnm1p (Drp1 orthologue) from mitochondria (25–27). Fis1p recruits Dnm1p to mitochondria through one of the two molecular adaptors, Mdv1p or Caf4p (28). Mdv1p, in addition, plays a post-recruitment function in the activation of Dnm1p (28–30).

STRUCTURAL DATA

Structural studies have given insight into how these proteins function at the molecular level. NMR (31) and crystallographic (32) studies have identified a tetratrico peptide repeat (TPR) domain in the cytosolic portion of Fis1. TPR motifs are packed spirals of anti-parallel α-helices. They are involved in protein–protein interactions and seem to be found most often in multi-protein complexes (33). In Fis1, the cytosolic domain folds into a helical bundle with a concave, hydrophobic surface that probably forms the binding site for a short peptide. Given the role of yeast Fis1p as the mitochondrial anchor for the fission machinery, it is quite plausible that the TPR domain of Fis1 serves as a scaffold for the assembly of a mammalian fission complex.

A crystallographic study of Mfn1 has likewise clarified the role of Mfn1 complexes in fusion (34). Cell hybrid studies on wild-type and Mfn-null cells indicate that Mfns are required for apposing mitochondria during fusion. A crystal structure of the C-terminal coiled coil (HR2) reveals that this interaction occurs via a dimeric, anti-parallel association of the HR2 regions (Fig. 2A). Because the transmembrane anchors of each Mfn1 molecule are on opposite sides of the coiled coil, this HR2 interaction provides a mechanism for tethering mitochondria. Tethered intermediates are found in other membrane trafficking systems prior to docking and membrane fusion. Indeed, mitochondria with Mfn1 lacking the GTPase domain cannot complete fusion, but aggregate with a uniform gap between them, as if trapped in a tethered intermediate.

CELLULAR FUNCTION

The central question of mitochondrial dynamics is what role it plays in mitochondrial and cellular functions. Clearly, fusion and fission control mitochondrial morphology. How different morphologies affect mitochondrial function is not clear yet, but specific changes in mitochondrial shape during animal development suggest that morphology and function are indeed closely linked. In addition, mounting evidence indicates that mitochondrial dynamics has roles beyond maintenance of morphology. Indeed, two key functions of mitochondria, electron transport and regulation of apoptosis, are affected by disruption of molecules involved in mitochondrial fusion and fission.

Oxygen electrode studies demonstrate that both endogenous and uncoupled respiration rates are reduced in Mfn-null cells and even more so in cells depleted of OPA1 by RNAi (OPA1–RNAi) (1). Attenuation of electron transport rates in

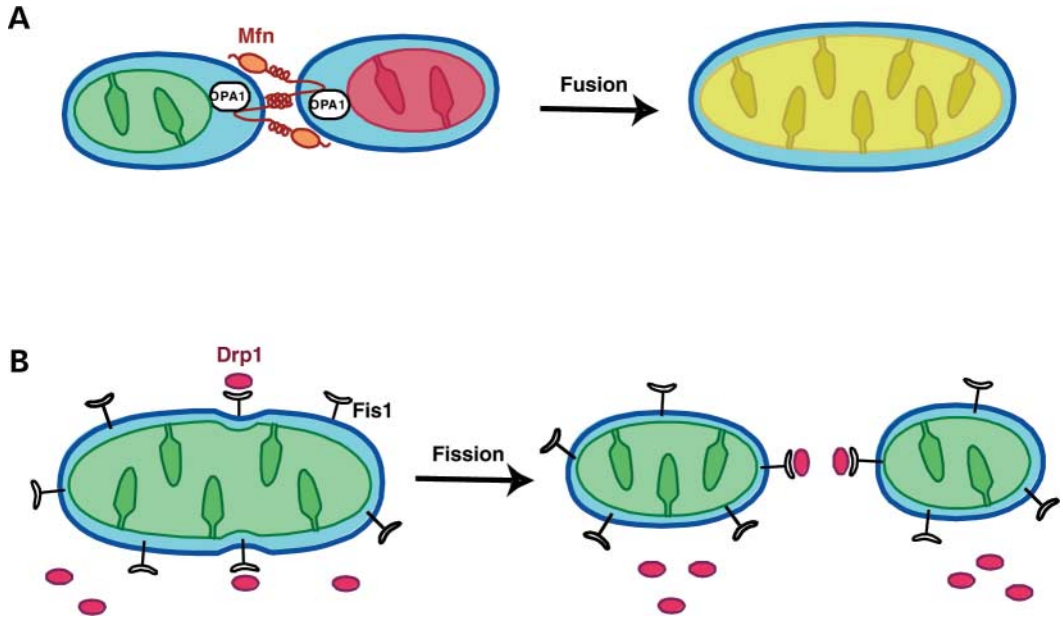

Figure 2. Mitochondrial fusion and fission molecules. (**A**) Fusion molecules. Mfn is a mitochondrial outer membrane protein with a cytosolic GTPase domain (orange oval) and two coiled coil regions (coils). The C-terminal coiled coil mediates oligomerization between Mfn molecules on adjacent mitochondria. OPA1 (white oval) is a GTPase in the intermembrane space. Mfns and OPA1 coordinate mitochondrial fusion, as shown by mixing of green and red matrix markers to produce yellow. (**B**) Fission molecules. Fis1 is localized uniformly to the mitochondrial outer membrane, whereas Drp1 is localized to the cytosol and punctate spots on mitochondria. Some of these spots are constriction sites that lead to mitochondrial fission. The mechanism of Drp1 recruitment to mitochondria is unclear.

respiration complexes I, III and IV contribute to this decrease. Normal respiration profiles are restored upon re-introduction of the fusion proteins, demonstrating reversibility of the defect. Both Mfn-null and OPA1–RNAi cells also exhibit reversible inhibition of cell growth and loss of mitochondrial membrane potential. How loss of fusion causes these functional defects remains to be resolved. One hypothesis is that mitochondrial fusion protects function by allowing rapid mixing of membrane and soluble contents, thereby ensuring that stochastic loss of materials is only transient. Without fusion, these losses become permanent and thus debilitating, resulting in cellular dysfunction (2).

In mammals, mitochondria play an important role in apoptotic cell death. Upon apoptosis induction, mitochondria often fragment (35). In one apoptotic pathway, Bax translocates from the cytosol to mitochondria and opens a pore in the outer membrane (36,37). Subsequent release of intermembrane space factors, including cytochrome *c*, results in caspase activation and ultimately, cell death (38). Both mitochondrial fission factors, Fis1 and Drp1, have now been implicated in this pathway (39).

Upon exposure to certain apoptotic stimuli, mitochondria accumulate more Drp1 puncta (35), which also co-localize with Bax (40). Drp1 co-localizes with Bax in punctate structures (40). Dominant-negative Drp1 and Drp1–RNAi prevent mitochondrial fragmentation during apoptosis, and interestingly, also reduce cell death, suggesting that Drp1 plays a role in apoptosis (35). However, Bax translocation still occurs, implying that Drp1 operates downstream of Bax mitochondrial localization (24). Fis1–RNAi also inhibits caspase-dependent cell death by several stimuli. This anti-apoptotic effect is greater than that of Drp1-RNAi and is accompanied by blockage of Bax translocation and cytochrome *c* release (24). This finding places Fis1 upstream of Bax mitochondrial localization and therefore suggests distinct functions for Fis1 and Drp1 during apoptosis.

It should be noted that absolute requirements for Fis1 and Drp1 in apoptosis cannot be determined without true null alleles. Also, evidence clearly indicates that alternative cell death pathways exist. In fact, fission may have protective effects in Ca^{++}-dependent apoptosis (41). Finally, it must be emphasized that mitochondrial fission occurs normally in healthy cells without leading to apoptosis.

As opposed to the fission molecules, mitochondrial fusion molecules seem to protect cells from apoptosis. Some apoptotic stimuli reduce mitochondrial fusion (42). Although co-overexpression of Mfn1 and Mfn2 can reduce cell death in the presence of apoptotic stimuli (43), OPA1-RNAi (24) and rat Mfn-RNAi (43) increase sensitivity to apoptotic stimuli. In fact, OPA1-RNAi even causes some spontaneous apoptosis (4).

Taken together, these studies suggest a model in which mitochondrial fission facilitates mammalian apoptosis, whereas mitochondrial fusion plays a protective role. The mechanisms involved are open to speculation. Mitochondrial fission may enhance apoptosis by increasing the availability of outer membrane surface area for pore formation. Because disruption of membrane continuity is inherent to the fission process, it is also possible that the fission machinery itself participates in outer membrane permeabilization. Mitochondrial fusion could protect from apoptosis by acting as a repair mechanism for outer membrane damage.

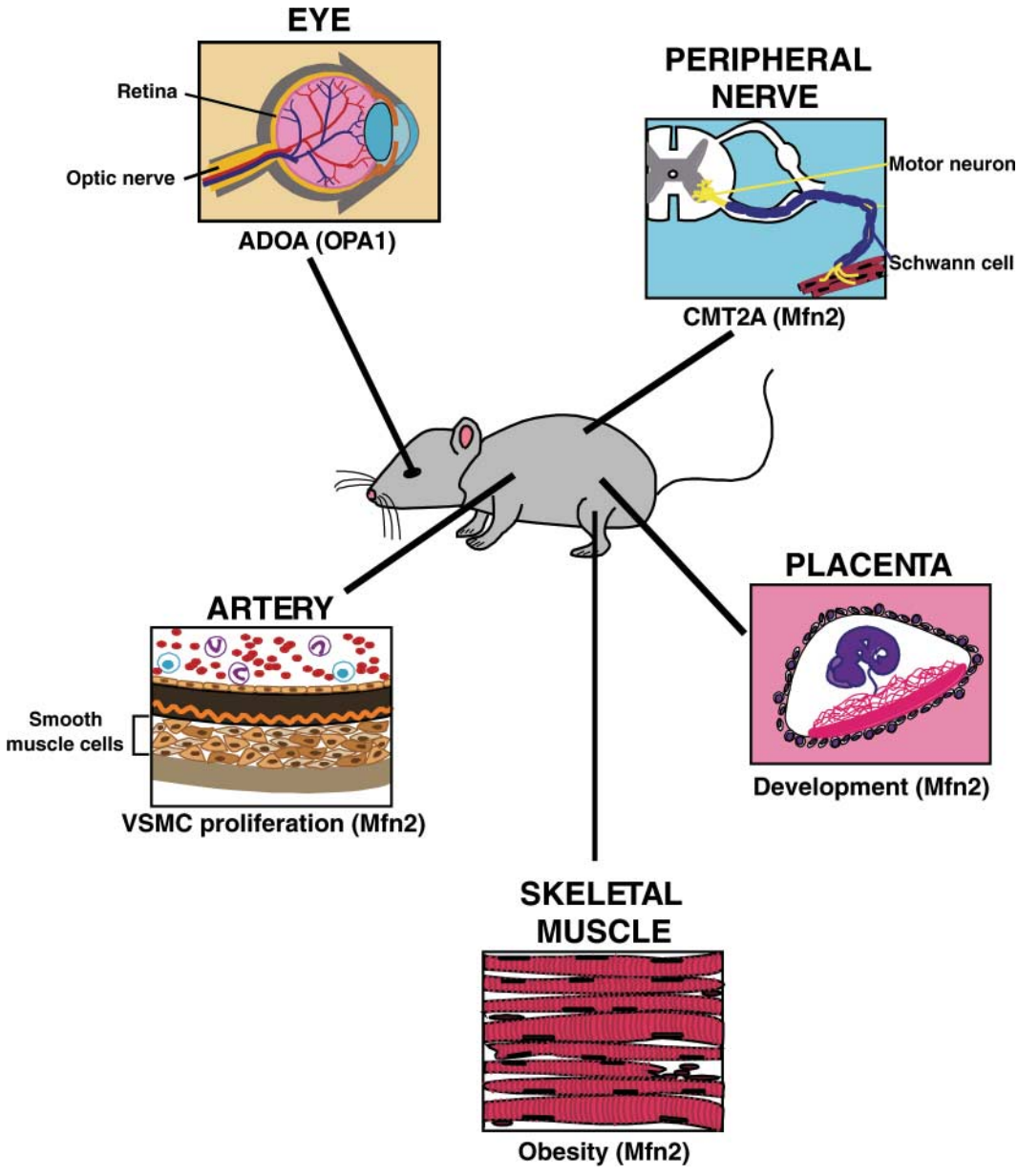

Figure 3. Mammalian tissues affected by defects in fusion molecules. OPA1 mutations cause defects in the retina and optic nerve. Mfn2 mutations affect the placenta, skeletal muscle, vascular smooth muscle cells and peripheral motor neurons.

PHYSIOLOGICAL IMPACT

How do the cellular functions of mitochondrial dynamics discussed previously influence the organism as a whole? Some insights have been gained from engineered and naturally occurring mutations in the Mfns and OPA1. These mutations have been implicated in developmental problems, neuropathies, obesity and vascular diseases (Fig. 3).

A targeted null mutation of either Mfn1 or Mfn2 results in mid-gestational lethality (2). Mfn1-null mice are easily distinguished by their severe developmental delay and distorted features. Mfn2-null embryos, however, are relatively normal until they die from defects of the placenta. These phenotypes indicate that mitochondrial fusion is required during development and that Mfn1 and Mfn2 are not completely redundant. Because the lethal nature of the null alleles preclude studying mitochondrial fusion in adult tissues, we have also engineered conditional alleles. Preliminary studies indicate that the nervous system is severely affected by the loss of Mfn2. Interestingly, a naturally occurring neuropathy is also associated with Mfn2 mutations.

Charcot–Marie–Tooth syndrome (CMT) is a cluster of hereditary peripheral motor neuropathies, some of which have concomitant sensory loss (44,45). CMT presents with progressive distal muscle weakness followed by muscular atrophy. Traditionally, clinical criteria have divided CMT into different subgroups. The two largest subgroups, CMT1 and CMT2, were thought to represent primary disorders in

Schwann cells and motor neurons, respectively. However, it is now clear that defects in one cell type can affect the other cell type and that different mutations in the same gene can cause both CMT1 and CMT2.

Mfn2 has been identified as the gene mutated in CMT2A, a dominantly inherited form of CMT (46). Thus far, 15 different mutations have been found in 21 families (46,47). Most mutations cluster in the GTPase domain or just upstream of it. The pathogenesis of CMT2A is unclear. Haploinsufficiency has been suggested, but Mfn2 $+/-$ mice exhibit no motor defects (2). Whether this is a true difference between mice and humans, possibly because of the vastly different axonal lengths of peripheral nerves, is yet to be determined. Alternatively, the mutant Mfn2 alleles may have dominant-negative or gain of function activity.

Another neuropathy linked to mitochondrial fusion is autosomal-dominant optic atrophy (ADOA) (48). This most frequent form of hereditary optic neuropathy is characterized by progressive loss of visual acuity, with wide ranges in both expressivity and penetrance. Histopathology indicates that primary degeneration of the retinal ganglia proceeds to atrophy of the optic nerve. The predominant gene responsible for ADOA has been identified as OPA1 (49,50). At least 83 different OPA1 mutations have been reported (51). Most represent gene truncations, with more than 50% found in the GTPase domain and the 3′ coiled coil region. Missense mutations are almost exclusively found in the GTPase domain. Some mutations may be semi-dominant, because one compound heterozygote patient has been reported to have more severe symptoms than the patient's simple-heterozygote parents and siblings (52). A family with a deleted OPA1 gene (53) and others with an exon 1 null mutation (52) indicate that haploinsufficiency may also be a cause.

How OPA1 mutations affect mitochondrial function in retinal ganglion cells is not clear yet, but recent studies provide tantalizing hints at mitochondria dysfunction. Clumped mitochondria in monocytes of affected patients indicate that mitochondrial morphology and/or localization is altered (50). Copy number of mitochondrial DNA (mtDNA) molecules was found to be lower in OPA1 patients (54). Finally, oxidative phosphorylation was shown to be deficient in calf muscle of patients (55).

It may not be a coincidence that two human diseases caused by defects in mitochondrial fusion genes are neurodegenerative disorders. Many mtDNA mutations also cause neurological problems. One common hypothesis for why neurons are so sensitive to changes in mitochondrial function is that neurons have high energy demands and/or require calcium regulation at distinct areas which may be quite distant from the cell body, namely axons or dendrites. Therefore, mitochondrial deficiencies may result in non-functional synapses, axonal degeneration and perhaps ultimately, cell death. This hypothesis has supporting evidence. Nerve growth factor (NGF) is a neurotrophin essential for the development and survival of sensory neurons. Interestingly, NGF induces mitochondrial migration down axons and accumulation at the NGF source (56). Similarly, time lapse microscopy demonstrates that a critical mitochondrial mass is required for dendritic spine development and maintenance (57). A mutation in the *Drosophila milton* gene prevents mitochondrial transport to synaptic terminals of photoreceptor cells, resulting in blind flies (58). Finally, Drosophila with mutations in Drp1 show reduced numbers of mitochondria at synapses and are unable to sustain neurotransmission under prolonged stimulation, probably due to improper mobilization of reserve pool vesicles (59). These findings strongly underscore the neuronal requirement for mitochondria in dendrites and axons. As discussed earlier, mitochondrial dynamics affects both mitochondrial localization and function and therefore would be expected to influence neuronal function.

Mfn2 may also play a role in two other disorders. Mfn2 was identified as a hypertension-suppressor gene by differential display (60). Rat cell culture studies and *in vivo* work show that overexpression of Mfn2 can inhibit vascular smooth muscle cell proliferation, although this effect appears independent of its mitochondrial function. A screen of differentially expressed genes from skeletal muscle of obese rats identified Mfn2 as a suppressor of obesity (61). Obese human subjects also expressed less Mfn2 than lean ones. Repression of Mfn2 expression in myotubes is associated with fragmentation of the mitochondrial network, reduction in glucose oxidation and diminished mitochondrial membrane potential (62).

CONCLUSION

Mitochondrial dynamics has been shown to play a critical role in determining mitochondrial morphology and function. In turn, developmental and physiological processes depend on these cellular functions. The best proof of this principle is the existence of human diseases caused by mutations in mitochondrial fusion genes.

Future directions in mammalian mitochondrial dynamics include identifying all molecular constituents of the fusion and fission machineries. This may require an *in vitro* assay for these activities, much like that already developed in yeast (63). Structural studies like those done for Mfn1 and Fis1 will be invaluable for elucidating the mechanism of these membrane remodelling events. Cell biological work is needed to reveal additional consequences of fusion and fission on mitochondrial and cellular functions. Two critical questions are how morphology affects function and how mitochondrial dynamics affects function independently of morphology. Finally, human and mouse genetic studies will be essential in uncovering the physiological functions of mitochondrial dynamics. Such genetic studies will in turn provide tools to clarify the biochemical interactions of these fusion and fission proteins and the basic cellular biology of mitochondrial dynamics.

Conflict of Interest statement. None declared.

REFERENCES

1. Chen, H., Chomyn, A. and Chan, D.C. (2005) Disruption of fusion results in mitochondrial heterogeneity and dysfunction. *J. Biol. Chem.*, **280**, 26185–26192.
2. Chen, H., Detmer, S.A., Ewald, A.J., Griffin, E.E., Fraser, S.E. and Chan, D.C. (2003) Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. *J. Cell Biol.*, **160**, 189–200.

3. Griparic, L., van der Wel, N.N., Orozco, I.J., Peters, P.J. and van der Bliek, A.M. (2004) Loss of the intermembrane space protein Mgm1/OPA1 induces swelling and localized constrictions along the lengths of mitochondria. *J. Biol. Chem.*, **279**, 18792–18798.
4. Olichon, A., Baricault, L., Gas, N., Guillou, E., Valette, A., Belenguer, P. and Lenaers, G. (2003) Loss of OPA1 perturbates the mitochondrial inner membrane structure and integrity, leading to cytochrome c release and apoptosis. *J. Biol. Chem.*, **278**, 7743–7746.
5. Smirnova, E., Griparic, L., Shurland, D.L. and van der Bliek, A.M. (2001) Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells. *Mol. Biol. Cell*, **12**, 2245–2256.
6. Yoon, Y., Krueger, E.W., Oswald, B.J. and McNiven, M.A. (2003) The mitochondrial protein hFis1 regulates mitochondrial fission in mammalian cells through an interaction with the dynamin-like protein DLP1. *Mol. Cell Biol.*, **23**, 5409–5420.
7. Stojanovski, D., Koutsopoulos, O.S., Okamoto, K. and Ryan, M.T. (2004) Levels of human Fis1 at the mitochondrial outer membrane regulate mitochondrial morphology. *J. Cell Sci.*, **117**, 1201–1210.
8. Cipolat, S., Martins de Brito, O., Dal Zilio, B. and Scorrano, L. (2004) OPA1 requires mitofusin 1 to promote mitochondrial fusion. *Proc. Natl Acad. Sci. USA*, **101**, 15927–15932.
9. Eura, Y., Ishihara, N., Yokota, S. and Mihara, K. (2003) Two mitofusin proteins, mammalian homologues of FZO, with distinct functions are both required for mitochondrial fusion. *J. Biochem. (Tokyo)*, **134**, 333–344.
10. Ishihara, N., Jofuku, A., Eura, Y. and Mihara, K. (2003) Regulation of mitochondrial morphology by membrane potential, and DRP1-dependent division and FZO1-dependent fusion reaction in mammalian cells. *Biochem. Biophys. Res. Commun.*, **301**, 891–898.
11. Legros, F., Lombes, A., Frachon, P. and Rojo, M. (2002) Mitochondrial fusion in human cells is efficient, requires the inner membrane potential, and is mediated by mitofusins. *Mol. Biol. Cell*, **13**, 4343–4354.
12. Rojo, M., Legros, F., Chateau, D. and Lombes, A. (2002) Membrane topology and mitochondrial targeting of mitofusins, ubiquitous mammalian homologs of the transmembrane GTPase Fzo. *J. Cell Sci.*, **115**, 1663–1674.
13. Santel, A., Frank, S., Gaume, B., Herrler, M., Youle, R.J. and Fuller, M.T. (2003) Mitofusin-1 protein is a generally expressed mediator of mitochondrial fusion in mammalian cells. *J. Cell Sci.*, **116**, 2763–2774.
14. Santel, A. and Fuller, M.T. (2001) Control of mitochondrial morphology by a human mitofusin. *J. Cell Sci.*, **114**, 867–874.
15. Olichon, A., Emorine, L.J., Descoins, E., Pelloquin, L., Brichese, L., Gas, N., Guillou, E., Delettre, C., Valette, A., Hamel, C.P. *et al.* (2002) The human dynamin-related protein OPA1 is anchored to the mitochondrial inner membrane facing the inter-membrane space. *FEBS Lett.*, **523**, 171–176.
16. Delettre, C., Griffoin, J.M., Kaplan, J., Dollfus, H., Lorenz, B., Faivre, L., Lenaers, G., Belenguer, P. and Hamel, C.P. (2001) Mutation spectrum and splicing variants in the OPA1 gene. *Hum. Genet.*, **109**, 584–591.
17. Satoh, M., Hamamoto, T., Seo, N., Kagawa, Y. and Endo, H. (2003) Differential sublocalization of the dynamin-related protein OPA1 isoforms in mitochondria. *Biochem. Biophys. Res. Commun.*, **300**, 482–493.
18. Herlan, M., Vogel, F., Bornhovd, C., Neupert, W. and Reichert, A.S. (2003) Processing of Mgm1 by the rhomboid-type protease Pcp1 is required for maintenance of mitochondrial morphology and of mitochondrial DNA. *J. Biol. Chem.*, **278**, 27781–27788.
19. McQuibban, G.A., Saurya, S. and Freeman, M. (2003) Mitochondrial membrane remodelling regulated by a conserved rhomboid protease. *Nature*, **423**, 537–541.
20. Sesaki, H., Southard, S.M., Hobbs, A.E. and Jensen, R.E. (2003) Cells lacking Pcp1p/Ugo2p, a rhomboid-like protease required for Mgm1p processing, lose mtDNA and mitochondrial structure in a Dnm1p-dependent manner, but remain competent for mitochondrial fusion. *Biochem. Biophys. Res. Commun.*, **308**, 276–283.
21. Herlan, M., Bornhovd, C., Hell, K., Neupert, W. and Reichert, A.S. (2004) Alternative topogenesis of Mgm1 and mitochondrial morphology depend on ATP and a functional import motor. *J. Cell Biol.*, **165**, 167–173.
22. Wong, E.D., Wagner, J.A., Scott, S.V., Okreglak, V., Holewinske, T.J., Cassidy-Stone, A. and Nunnari, J. (2003) The intramitochondrial dynamin-related GTPase, Mgm1p, is a component of a protein complex that mediates mitochondrial fusion. *J. Cell Biol.*, **160**, 303–311.
23. Zhu, P.P., Patterson, A., Stadler, J., Seeburg, D.P., Sheng, M. and Blackstone, C. (2004) 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.
24. Lee, Y.J., Jeong, S.Y., Karbowski, M., Smith, C.L. and Youle, R.J. (2004) Roles of the mammalian mitochondrial fission and fusion mediators Fis1, Drp1, and Opa1 in apoptosis. *Mol. Biol. Cell*, **15**, 5001–5011.
25. Fekkes, P., Shepard, K.A. and Yaffe, M.P. (2000) Gag3p, an outer membrane protein required for fission of mitochondrial tubules. *J. Cell Biol.*, **151**, 333–340.
26. Mozdy, A.D., McCaffery, J.M. and Shaw, J.M. (2000) Dnm1p GTPase-mediated mitochondrial fission is a multi-step process requiring the novel integral membrane component Fis1p. *J. Cell Biol.*, **151**, 367–380.
27. Tieu, Q. and Nunnari, J. (2000) Mdv1p is a WD repeat protein that interacts with the dynamin-related GTPase, Dnm1p, to trigger mitochondrial division. *J. Cell Biol.*, **151**, 353–366.
28. Griffin, E.E., Graumann, J. and Chan, D.C. (2005) The WD40 protein Caf4p is a component of the mitochondrial fission machinery and recruits Dnm1p to mitochondria. *J. Cell Biol.*, **170**, 237–248.
29. Cerveny, K.L. and Jensen, R.E. (2003) The WD-repeats of Net2p interact with Dnm1p and Fis1p to regulate division of mitochondria. *Mol. Biol. Cell*, **14**, 4126–4139.
30. Tieu, Q., Okreglak, V., Naylor, K. and Nunnari, J. (2002) 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.
31. Suzuki, M., Jeong, S.Y., Karbowski, M., Youle, R.J. and Tjandra, N. (2003) The solution structure of human mitochondria fission protein Fis1 reveals a novel TPR-like helix bundle. *J. Mol. Biol.*, **334**, 445–458.
32. Dohm, J.A., Lee, S.J., Hardwick, J.M., Hill, R.B. and Gittis, A.G. (2004) Cytosolic domain of the human mitochondrial fission protein fis1 adopts a TPR fold. *Proteins*, **54**, 153–156.
33. D'Andrea, L.D. and Regan, L. (2003) TPR proteins: the versatile helix. *Trends Biochem. Sci.*, **28**, 655–662.
34. Koshiba, T., Detmer, S.A., Kaiser, J.T., Chen, H., McCaffery, J.M. and Chan, D.C. (2004) Structural basis of mitochondrial tethering by mitofusin complexes. *Science*, **305**, 858–862.
35. Frank, S., Gaume, B., Bergmann-Leitner, E.S., Leitner, W.W., Robert, E.G., Catez, F., Smith, C.L. and Youle, R.J. (2001) The role of dynamin-related protein 1, a mediator of mitochondrial fission, in apoptosis. *Dev. Cell*, **1**, 515–525.
36. Wolter, K.G., Hsu, Y.T., Smith, C.L., Nechushtan, A., Xi, X.G. and Youle, R.J. (1997) Movement of Bax from the cytosol to mitochondria during apoptosis. *J. Cell Biol.*, **139**, 1281–1292.
37. Suzuki, M., Youle, R.J. and Tjandra, N. (2000) Structure of Bax: coregulation of dimer formation and intracellular localization. *Cell*, **103**, 645–654.
38. Green, D.R. and Reed, J.C. (1998) Mitochondria and apoptosis. *Science*, **281**, 1309–1312.
39. Perfettini, J.L., Roumier, T. and Kroemer, G. (2005) Mitochondrial fusion and fission in the control of apoptosis. *Trends Cell Biol.*, **15**, 179–183.
40. Karbowski, M., Lee, Y.J., Gaume, B., Jeong, S.Y., Frank, S., Nechushtan, A., Santel, A., Fuller, M., Smith, C.L. and Youle, R.J. (2002) Spatial and temporal association of Bax with mitochondrial fission sites, Drp1, and Mfn2 during apoptosis. *J. Cell Biol.*, **159**, 931–938.
41. Szabadkai, G., Simoni, A.M., Chami, M., Wieckowski, M.R., Youle, R.J. and Rizzuto, R. (2004) Drp-1-dependent division of the mitochondrial network blocks intraorganellar Ca2+ waves and protects against Ca2+-mediated apoptosis. *Mol. Cell*, **16**, 59–68.
42. Karbowski, M., Arnoult, D., Chen, H., Chan, D.C., Smith, C.L. and Youle, R.J. (2004) 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.
43. Sugioka, R., Shimizu, S. and Tsujimoto, Y. (2004) Fzo1, a protein involved in mitochondrial fusion, inhibits apoptosis. *J. Biol. Chem.*, **279**, 52726–52734.
44. Young, P. and Suter, U. (2003) The causes of Charcot–Marie–Tooth disease. *Cell. Mol. Life Sci.*, **60**, 2547–2560.
45. Shy, M.E. (2004) Charcot–Marie–Tooth disease: an update. *Curr. Opin. Neurol.*, **17**, 579–585.
46. Zuchner, S., Mersiyanova, I.V., Muglia, M., Bissar-Tadmouri, N., Rochelle, J., Dadali, E.L., Zappia, M., Nelis, E., Patitucci, A., Senderek, J. *et al.* (2004) Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot–Marie–Tooth neuropathy type 2A. *Nat. Genet.*, **36**, 449–451.

47. Kijima, K., Numakura, C., Izumino, H., Umetsu, K., Nezu, A., Shiiki, T., Ogawa, M., Ishizaki, Y., Kitamura, T., Shozawa, Y. *et al.* (2005) Mitochondrial GTPase mitofusin 2 mutation in Charcot–Marie–Tooth neuropathy type 2A. *Hum. Genet.*, **116**, 23–27.
48. Delettre, C., Lenaers, G., Pelloquin, L., Belenguer, P. and Hamel, C.P. (2002) OPA1 (Kjer type) dominant optic atrophy: a novel mitochondrial disease. *Mol. Genet. Metab.*, **75**, 97–107.
49. Alexander, C., Votruba, M., Pesch, U.E., Thiselton, D.L., Mayer, S., Moore, A., Rodriguez, M., Kellner, U., Leo-Kottler, B., Auburger, G. *et al.* (2000) OPA1, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28. *Nat. Genet.*, **26**, 211–215.
50. Delettre, C., Lenaers, G., Griffoin, J.M., Gigarel, N., Lorenzo, C., Belenguer, P., Pelloquin, L., Grosgeorge, J., Turc-Carel, C., Perret, E. *et al.* (2000) Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. *Nat. Genet.*, **26**, 207–210.
51. Ferre, M., Amati-Bonneau, P., Tourmen, Y., Malthiery, Y. and Reynier, P. (2005) eOPA1: an online database for OPA1 mutations. *Hum. Mutat.*, **25**, 423–428.
52. Pesch, U.E., Leo-Kottler, B., Mayer, S., Jurklies, B., Kellner, U., Apfelstedt-Sylla, E., Zrenner, E., Alexander, C. and Wissinger, B. (2001) OPA1 mutations in patients with autosomal dominant optic atrophy and evidence for semi-dominant inheritance. *Hum. Mol. Genet.*, **10**, 1359–1368.
53. Marchbank, N.J., Craig, J.E., Leek, J.P., Toohey, M., Churchill, A.J., Markham, A.F., Mackey, D.A., Toomes, C. and Inglehearn, C.F. (2002) Deletion of the OPA1 gene in a dominant optic atrophy family: evidence that haploinsufficiency is the cause of disease. *J. Med. Genet.*, **39**, e47.
54. Kim, J.Y., Hwang, J.M., Ko, H.S., Seong, M.W., Park, B.J. and Park, S.S. (2005) Mitochondrial DNA content is decreased in autosomal dominant optic atrophy. *Neurology*, **64**, 966–972.
55. Lodi, R., Tonon, C., Valentino, M.L., Iotti, S., Clementi, V., Malucelli, E., Barboni, P., Longanesi, L., Schimpf, S., Wissinger, B. *et al.* (2004) Deficit of *in vivo* mitochondrial ATP production in OPA1-related dominant optic atrophy. *Ann. Neurol.*, **56**, 719–723.
56. Chada, S.R. and Hollenbeck, P.J. (2004) Nerve growth factor signaling regulates motility and docking of axonal mitochondria. *Curr. Biol.*, **14**, 1272–1276.
57. Li, Z., Okamoto, K., Hayashi, Y. and Sheng, M. (2004) The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. *Cell*, **119**, 873–887.
58. Stowers, R.S., Megeath, L.J., Gorska-Andrzejak, J., Meinertzhagen, I.A. and Schwarz, T.L. (2002) Axonal transport of mitochondria to synapses depends on milton, a novel Drosophila protein. *Neuron*, **36**, 1063–1077.
59. Verstreken, P., Ly, C.V., Venken, K.J., Koh, T.W., Zhou, Y. and Bellen, H.J. (2005) Synaptic mitochondria are critical for mobilization of reserve pool vesicles at Drosophila neuromuscular junctions. *Neuron*, **47**, 365–78.
60. Chen, K.H., Guo, X., Ma, D., Guo, Y., Li, Q., Yang, D., Li, P., Qiu, X., Wen, S., Xiao, R.P. *et al.* (2004) Dysregulation of HSG triggers vascular proliferative disorders. *Nat. Cell Biol.*, **6**, 872–883.
61. Bach, D., Pich, S., Soriano, F.X., Vega, N., Baumgartner, B., Oriola, J., Daugaard, J.R., Lloberas, J., Camps, M., Zierath, J.R. *et al.* (2003) Mitofusin-2 determines mitochondrial network architecture and mitochondrial metabolism. A novel regulatory mechanism altered in obesity. *J. Biol. Chem.*, **278**, 17190–17197.
62. Pich, S., Bach, D., Briones, P., Liesa, M., Camps, M., Testar, X., Palacin, M. and Zorzano, A. (2005) The Charcot–Marie–Tooth type 2A gene product, Mfn2, up-regulates fuel oxidation through expression of OXPHOS system. *Hum. Mol. Genet.*, **14**, 1405–1415.
63. Meeusen, S., McCaffery, J.M. and Nunnari, J. (2004) Mitochondrial fusion intermediates revealed *in vitro*. *Science*, **305**, 1747–1752.