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Minireview

Mitochondrial morphology and distribution in mammalian cells

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Abstract

It is now appreciated that mitochondria form tubular networks that adapt to the requirements of the cell by undergoing changes in their shape through fission and fusion. Proper mitochondrial distribution also appears to be required for ATP delivery and calcium regulation, and, in some cases, for cell development. While we now realise the great importance of mitochondria for the cell, we are only beginning to work out how these organelles undergo the drastic morphological changes that are essential for cellular function. Of the few known components involved in shaping mitochondria, some have been found to be essential to life and their gene mutations are linked to neurological disorders, while others appear to be recruited in the activation of cell death pathways. Here we review our current understanding of the functions of the main players involved in mitochondrial fission, fusion and distribution in mammalian cells.

Keywords: apoptosis; cytoskeleton; fission; fusion; mitochondria.

Introduction

Mitochondria are dynamic organelles with morphological variations intricately linked to many cellular processes, including development, cell cycle progression and death. These variations reflect the various roles of mitochondria within the cell, which include energy generation, metabolism and Ca^{2+} homeostasis. In most mammalian cells, mitochondria exist as a branched reticular network that radiates from the nucleus, although the dynamic nature of their morphology is reflected by differences in number, size and positioning within different cell lines, tissues and organisms (Figure 1). Dramatic alterations of the mitochondrial network allow specialised cells to cater to differing energy requirements and provide a means for environmental sensing. For example, an important early step in spermatogenesis is the wrapping of fused mitochondria at the base of the flagellum to supply ATP for movement (Hales and Fuller, 1997), whereas in neuronal cells, mitochondria are transported along the length of the axon to ensure adequate ATP provision and/or regulation of Ca^{2+} levels throughout the cell (Hollenbeck and Saxton, 2005). Likewise, recent work suggests that mitochondria move into new areas of the neuron, where they facilitate spine and synapse formation (Li et al., 2004). Pancreatic acinar cells also appear to contain populations of mitochondria situated in distinct cellular regions for buffering of Ca^{2+} waves and for modulating ATP-mediated insulin secretion (Tinel et al., 1999).

The maintenance and regulation of steady-state mitochondrial morphology relies on both an association with the cytoskeleton and a balance between opposing and ongoing fission and fusion events (Figure 2). Several proteins have now been identified that are responsible for mediating the events of mitochondrial fission and fusion (Table 1), with much of the initial research involving the yeast *Saccharomyces cerevisiae*. However, the precise molecular mechanism and execution of the mitochondrial fusion and fission processes are not entirely understood, most likely because additional factors are yet to be identified. Furthermore, analyses of mammalian mitochondrial morphology components controlling the fission, fusion and distribution pathways have revealed important distinctions between the systems. For instance, several of the yeast components identified have no equivalents in mammals (Okamoto and Shaw, 2005), probably because yeast divide by budding and track their mitochondria along actin fibres, while mammalian mitochondria predominantly rely on the microtubule network for distribution. This review highlights what is known at present about the components involved in shaping mitochondria in mammals and their links to apoptosis and human disease.

Mitochondrial fusion machinery

The fusion of mitochondria and subsequent mixing of the individual compartments ensures continuity of the contents and has been proposed to act as a mechanism to transmit energy and calcium signals (Skulachev, 2001). These mitochondrial fusion events need to be highly regulated, as coordinated fusion of the two membranes must preserve the integrity of the distinct mitochondrial compartments. Insights into the steps required for mitochondrial fusion have come from the identification of several key mediators in this process, as well as the development of specialised *in vivo* and *in vitro* assays.

The first reported mediator of mitochondrial fusion was identified in the fruit fly *Drosophila melanogaster*. The

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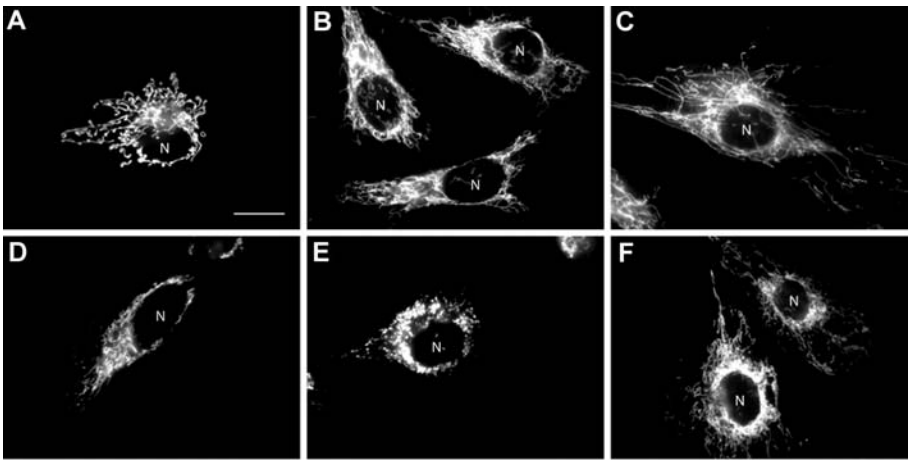

Figure 1 Mitochondrial morphology in different cell types.
Mitochondria from live tissue culture cells were stained with MitoTracker Red CMXRos and visualised by epifluorescence. (A) COS-7 monkey kidney fibroblasts, (B) HeLa human epithelial carcinoma cells, (C) S90 human skin fibroblasts, (D) 143BTK$^-$ human osteosarcoma cells, (E) 143BTK$^-$-87 ρ^0 human osteosarcoma cells and (F) C2C12 mouse myoblasts. The scale bar in panel (A) indicates 20 μm and is representative for all images. N, nucleus.

product of the *fzo* (fuzzy onion) gene was found to be required for developmentally regulated mitochondrial fusion events during spermatogenesis (Hales and Fuller, 1997). Two Fzo mammalian homologues, termed mitofusins (Mfn) 1 and 2, are found in the mitochondrial outer membrane and span the membrane twice, thereby exposing an N-terminal GTPase domain and two predicted coiled-coil regions to the cytosol (Rojo et al., 2002; Figure 2). Mouse knockouts emphasised the physiological relevance of the mitofusins, as the absence of either leads to embryonic lethality (Chen et al., 2003). Overexpression of Mfn proteins leads to extensive mitochon-

Table 1 Mammalian and yeast factors involved in mitochondrial fusion, fission and distribution.

Process	Mammals	Yeast	Localisation	Proposed function
Mitochondrial fusion	Mfn1/Mfn2	Fzo1p	Mitochondrial outer membrane	Integral membrane GTPases involved in tethering of mitochondria for fusion; Mfn2 linked to CMT type 2A disease
	OPA1	Mgm1p	Intermembrane space/inner membrane	Inner membrane fusion and/or cristae remodelling; linked to the disease ADOA
	–	Ugo1p	Intermembrane space	Adapter molecule for Mgm1p and Fzo1p in yeast
Mitochondrial fission	Drp1	Dnm1p	Cytosol/mitochondrial association	Dynamin-like GTPase oligomerises around mitochondrial tubules during fission
	Fis1	Fis1p	Mitochondrial outer membrane	Integral membrane protein that recruits Drp1 to mitochondria for fission
	–	Mdv1p	Co-localisation with Dnm1p	WD40 repeat protein mediating Dnm1p-Fis1p interaction
	MTP18	–	Intramitochondrial membrane protein	Role in maintaining mitochondrial fission/morphological dynamics
	Endophilin B1	–	Cytosol/mitochondrial association	Role in maintaining mitochondrial fission/morphological dynamics
Mitochondrial distribution	Miro-1/Miro-2	Gem1p	Mitochondrial outer membrane	Integral membrane Rho GTPase with role in maintenance of mitochondrial morphology
	Milt1/Milt2	–	Mitochondrial outer membrane	Coiled-coil proteins that interact with kinesin heavy chain; limited similarity to Huntington-associated protein 1
	Syntabulin	–	Mitochondrial membrane association	Anterograde transport of mitochondria in neurons; interacts with kinesin 1
	KBP	–	Mitochondrial outer membrane	Mediates mitochondrial trafficking; interacts with kinesin 3

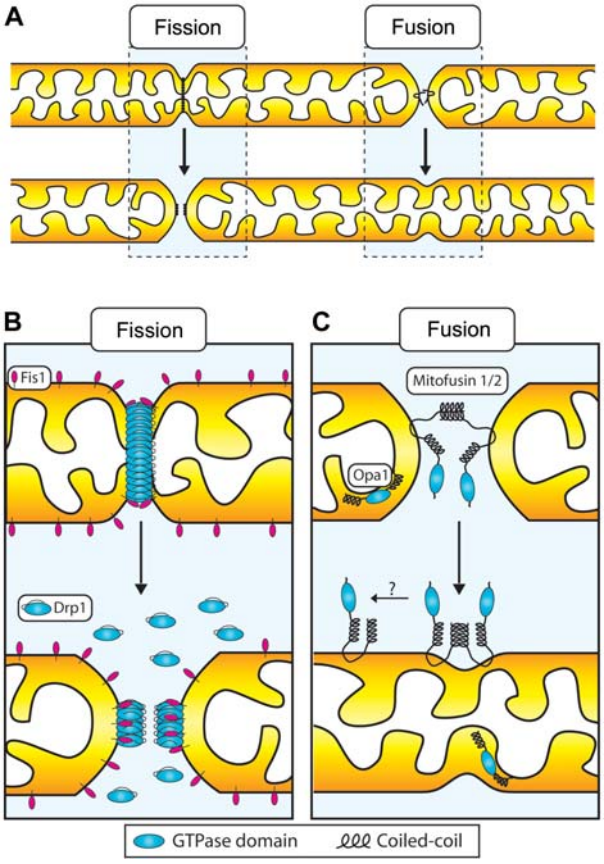

Figure 2 Model for mitochondrial fission and fusion in mammalian cells.
(A) An individual mitochondrial tubule can undergo constriction and subsequent fission (left) or two tubules can tether before fusion of the outer and inner membranes and mixing of compartments (right). (B) Magnification of the area showing the main components known to be involved in fission: Fis1, the outer membrane receptor-like protein, and Drp1, which polymerises around mitochondrial constriction sites to facilitate membrane scission. (C) During fusion, mitochondria tether to one another through an interaction between opposing mitofusins. GTP hydrolysis facilitates the fusion process. Opa1 in the intermembrane space is thought to facilitate inner membrane fusion and/or remodelling of cristae. Unknown factors are presumably involved in uncoiling Mfn1/2 following fusion.

drial branching and eventual perinuclear clustering of mitochondria (Santel and Fuller, 2001; Legros et al., 2002; Rojo et al., 2002; Chen and Chan, 2004). Alternatively, knockdown or loss of Mfn1 or Mfn2 results in fragmentation of the mitochondrial network, although the individual phenotypes are distinct (Chen et al., 2003; Eura et al., 2003).

Mitochondrial fusion assays showed that mitofusins must be present on adjacent mitochondrial membranes for membrane fusion to occur (Eura et al., 2003; Koshiba et al., 2004). Indeed the structure of the C-terminal end of Mfn1 revealed that it forms a 95-Å dimeric, antiparallel coiled-coil (Koshiba et al., 2004) similar to that of other membrane fusion proteins. Based on these data, it appears that this region is required for membrane tethering of mitochondria prior to downstream events leading to fusion (Koshiba et al., 2004). This tethering appears to rely on the GTPase domain in Mfn proteins (Ishihara et al., 2004). However, the mechanism by which the membranes become closely apposed and fused following tethering remains to be elucidated. Presumably, a mechanism to allow uncoiling of the mitofusins after membrane fusion is also required, although this has yet to be analysed. In addition to the cytosolic domains, the short inter-membrane space loop has been shown to be crucial for function of the yeast Mfn counterpart (Fzo1p), which forms contact sites between the mitochondrial outer and inner membranes (Fritz et al., 2001). Thus, fusion of the outer membrane can be tightly linked to that of the inner membrane.

As yet, only one additional component, OPA1, has been shown to be involved in mitochondrial fusion in mammalian cells. While OPA1 has been localised to the intermembrane space and is thought to associate with the inner membrane (Olichon et al., 2002; Griparic et al., 2004), certain isoforms display alternate sublocalisations (Satoh et al., 2003). Mutations in the yeast counterpart of OPA1 (termed Mgm1) result in mitochondrial fragmentation, loss of mtDNA and blocked fusion (Wong et al., 2000). However, the function of the human equivalent, OPA1, has yet to be precisely determined. RNA interference of OPA1 causes fragmentation of the mitochondrial network (Olichon et al., 2003; Griparic et al., 2004; Lee et al., 2004) and disrupts mitochondrial fusion in *in vivo* assays (Chen et al., 2005; Cipolat et al., 2004). However, over-expression of OPA1 also induces fragmentation of the mitochondrial network in certain cultured cell lines (Misaka et al., 2002; Griparic et al., 2004) while increasing tubulation in others (Cipolat et al., 2004). The fragmentation observed appears to result from increased fission rather than decreased fusion, and may reflect an imbalance between OPA1 isoforms (Chen et al., 2005). In addition to a role in mitochondrial fusion, OPA1 has been linked to cristae maintenance/formation and therefore may have an additional role in mitochondrial shape maintenance (Olichon et al., 2003; Griparic et al., 2004).

Mitochondrial fission machinery

Mitochondria cannot be created *de novo*, but instead replicate by a process of recruitment of proteins to pre-existing organelles. An active fission apparatus is therefore required to promote transmission of mitochondria to dividing cells, ensuring progeny cell survival (Figure 2). Mitochondrial proliferation and division take place in cells undergoing differentiation and growth in response to the ATP requirements of the cell/tissue, and play a role in the replacement of damaged and ageing organelles. The first mediator of mitochondrial fission, Dnm1, was uncovered in a screen for yeast mutants with defective mitochondrial morphology (Otsuga et al., 1998). A member of the dynamin family of GTPases (Praefcke and McMahon, 2004), its mammalian counterpart is termed dynamin-related protein (Drp1) or dynamin-like protein (Dlp1) (Smirnova et al., 1998). Over-expression of Drp1 has little effect on mitochondrial morphology in cultured cells, most likely due to an excess pool of protein within the cytosol (Smirnova et al., 1998). On the other hand, knockdown of Drp1 (Lee et al., 2004) or over-expression of a dominant-negative Drp1 mutant containing a dis-

rupted GTPase domain (Drp^{K38A}) blocks fission and remodels mitochondria into an array of interconnected tubules (Smirnova et al., 1998, 2001). Time-lapse imaging of GFP-tagged Drp1 and its counterparts revealed that it associates into punctae at the mitochondrial surface and cycles on and off the organelle at sites of fission (Smirnova et al., 2001; Okamoto and Shaw, 2005). Furthermore, the occurrence of a fission event in yeast was found to be dependent on constriction of the mitochondrial tubule and the presence of a dynamic Dnm1p 'ring' structure (Legesse-Miller et al., 2003). Ring-like structures of oligomerised Drp1 observed *in vitro* (Smirnova et al., 2001; Yoon et al., 2001) are similar to those formed by Dnm1 in yeast, which have a diameter that approximately corresponds to a constricted mitochondrial segment (Ingerman et al., 2005).

In yeast, genetic approaches to identify Dnm1p partner proteins led to the identification of Fis1p (Fission 1 protein, also termed Mdv2p or Gag2p) and Mdv1p (mitochondrial division 1 protein, also termed Fis2p, Net2p, or Gag3p) (Fekkes et al., 2000; Mozdy et al., 2000; Tieu and Nunnari, 2000; Cerveny et al., 2001). Fis1p is an evolutionarily conserved 17-kDa protein that is uniformly distributed along the mitochondrial outer membrane. Its identification in yeast represented the first defined mitochondrial membrane protein that participates in fission events (Okamoto and Shaw, 2005). Over-expression of human Fis1 causes fragmentation of the mitochondrial network due to accelerated mitochondrial fission events (James et al., 2003; Yoon et al., 2003; Stojanovski et al., 2004). Conversely, depletion of hFis1 by RNA interference induces an elongation of mitochondrial tubules consistent with a block in mitochondrial fission (Stojanovski et al., 2004). When hFis1 is co-expressed with a dominant-negative mutant of Drp1 ($Drp1^{K38A}$), the rate of mitochondrial fragmentation is reduced, indicating participation of hFis1 and Drp1 in a common fission pathway that regulates mitochondrial morphology (James et al., 2003; Yoon et al., 2003; Stojanovski et al., 2004). Taken together, these data support a mechanism whereby the levels of hFis1 on the mitochondrial outer membrane regulate the rate of mitochondrial fission events, in conjunction with Drp1. Fis1 forms a TPR-like helix bundle indicating sites for potential protein interactions (Suzuki et al., 2003). Unlike the yeast system, however, it is unclear whether Fis1 is important for Drp1 docking onto mitochondria, since no change in Drp1 distribution was found in cells depleted of Fis1 (Lee et al., 2004).

While yeast Mdv1p co-localises with Dnm1p at punctate structures along the mitochondrial outer membrane and has been shown to physically interact with both Dnm1 and Fis1 (Okamoto and Shaw, 2005), orthologues in higher eukaryotes have not been found. However, two additional proteins, MTP18 (Tondera et al., 2005) and endophilin B1 (Karbowski et al., 2004), have been implicated in mammalian mitochondrial fission, although their roles are less clearly defined.

Mitochondrial distribution

Trafficking of mitochondria throughout the cell is thought to be important for cellular function by placing them in locations with high energy requirements. This was elegantly demonstrated in neuronal dendrites, whereby the distribution of mitochondria was shown to be essential for their development and plasticity (Li et al., 2004). Perturbation of Drp1 or Opa1 affected the number of dendritic mitochondria, which in turn affected the number and plasticity of spines and synapses (Li et al., 2004). Likewise, the reduction in presynaptic mitochondria stemming from defective intracellular transport results in neurons defective in synaptic transmission (Stowers et al., 2002).

In mammalian cells, mitochondria are predominantly transported along the microtubule network (Hollenbeck and Saxton, 2005), while in budding yeast, they depend mainly on actin microfilaments for proper distribution (Okamoto and Shaw, 2005). Consequently, *S. cerevisiae* and higher eukaryotes have evolved a separate complement of proteins for mitochondrial distribution (Table 1). In mammals, both kinesin and dynein members have been found to be involved in anterograde and retrograde transport of mitochondria along microtubules. Analysis of mouse embryonic stem cells disrupted of the essential *kif5B* gene, which encodes the kinesin-1 family member KIF5B, results in perinuclear clustering of mitochondria (Tanaka et al., 1998). Chemical-induced depolymerisation of the microtubules caused redispersal of mitochondria, indicating that dynein-mediated retrograde transport was still taking place (Tanaka et al., 1998). However, disruption of the dynein-dynactin motor complex by antibody injection or subunit over-expression also causes a disruption of normal mitochondrial morphology and collapse towards the nucleus (Varadi et al., 2004). Over-expression of Drp1 in cells disrupted for dynein function is able to overcome the collapsed morphology, leading to the proposal that dynein may also mediate the recruitment of Drp1 to mitochondria for the fission process (Varadi et al., 2004). Interestingly, an actin-mediated recruitment of Drp1 to mitochondria for fission has also been demonstrated (De Vos et al., 2005) and there is evidence that association and short-range movement of mitochondria in mammalian cells may involve additional contacts and transport along actin fibrils (Knowles et al., 2002; Hollenbeck and Saxton, 2005). Such differences may reflect different cellular pathways for regulation of mitochondrial morphology.

Little is known about the mechanisms by which mitochondria are connected to cytoskeletal motors and whether they involve linkers, adaptors or direct contact. Several proteins have been identified that interact both with mitochondria and kinesins, but little is known about their exact functions. The mitochondrial-associated protein syntabulin was implicated in the process of anterograde mitochondrial transport along microtubules in neurons (Cai et al., 2005). Syntabulin was initially isolated as a protein that directly interacts with the kinesin-1 KIF5B to mediate transfer of syntaxin-containing vesicles to neuronal processes. Importantly, a decrease in syntabulin levels by siRNA or inhibition by dominant-negative mutants reduced the distribution of mitochondria to neuronal processes (Cai et al., 2005). Likewise, the mitochondrial localised KBP (KIF1 binding protein) was identified as a protein that binds to the kinesin-3 KIF1Bα

(Wozniak et al., 2005). Inactivation of KBP caused aggregation of mitochondria, yet did not affect KIF1Bα localisation to mitochondria, indicating that instead of acting as a linker, KBP may function in a regulatory role (Wozniak et al., 2005).

The *milton* gene was originally identified in a *Drosophila* photoreceptor mutant screen that showed that mutation of *milton* prevented mitochondrial trafficking to synaptic terminals and axons in neurons, causing blindness (Stowers et al., 2002). HuMilt1 (OIP106) and HuMilt2 (GRIF-1/OIP98) are the mammalian orthologues of *Drosophila* Milton (Stowers et al., 2002; Brickley et al., 2005). Like Milton, these orthologues are coiled-coil proteins found at the mitochondrial outer membrane, where they interact with kinesin. Interestingly, Milton family members also share homology with Huntington-associated protein (HAP-1), which is thought to act in intracellular trafficking (Stowers et al., 2002). Indeed, HuMilt1 and HuMilt2 have been found to associate with several other proteins, suggesting that they have multiple roles in the cell, likely linking both vesicular and mitochondrial cargoes to microtubule transport.

Miro-1 and Miro-2 are Rho-like GTPases that localise to mitochondria and contain two putative GTPase domains flanking two EF-hand domains (Fransson et al., 2003). While over-expression of the proteins does not affect mitochondrial morphology, over-expression of a constitutively active GTPase mutant (Miro-1/Val-13) results in perinuclear clustering of mitochondria, with no effect on cytoskeletal components (Fransson et al., 2003). Likewise, mutations in the *Drosophila* orthologue, dMiro, result in abnormal distribution of mitochondria to neurons and are lethal (Guo et al., 2005). The *S. cerevisiae* orthologue of the Miro proteins, Gem1p, has also been implicated in the maintenance of mitochondrial morphology in a manner that is independent of fission and fusion events (Frederick et al., 2004). The regulation of actin structures by GTPases of the Rho family in mammalian cells makes it tempting to speculate that the Miro proteins may affect mitochondrial connections with the cytoskeleton. Correspondingly, neuronal transport of mitochondria is arrested in *Drosophila* dMiro mutants in a manner reminiscent of a block in anterograde transport processes (Guo et al., 2005). Owing to the presence of the calcium-binding EF-hand motif, it has been speculated that the protein plays a regulatory role in mitochondrial shaping in response to environmental signals (Frederick et al., 2004).

Mitochondrial morphology in disease

It has become clear that the maintenance of mitochondrial morphology is linked to proper mitochondrial function and this is exemplified by mutations in the genes encoding mammalian mitochondrial fusion proteins causing human disease. Mutations in Mfn2 have been found to cause Charcot-Marie-Tooth (CMT) type 2A disease, the axonal form of a common inherited peripheral neuropathy (Züchner et al., 2004; Kijima et al., 2005). Correlating with evidence available thus far for mitofusin function, the majority of the mutations lie within or just upstream of the GTPase domain or within the coiled-coil domains required for protein interactions (Züchner et al., 2004; Kijima et al., 2005). An alternate form of axonal CMT neuropathy with associated optic atrophy, known as hereditary motor and sensory neuropathy type VI (HMSN VI), has also been linked to mutations in Mfn2 (Züchner et al., 2006). Interestingly, Chen et al. (2003) observed that mitochondria in mitofusin-deficient cells show defects in mobility within the cell, which could be potentially damaging to nerve cells that require mitochondrial transport along the length of the axon.

Mutations in *OPA1* give rise to a common hereditary form of optic neuropathy, autosomal dominant optic atrophy (ADOA, or Kjer disease), in which the death of retinal ganglion cells leads to a progressive loss of vision (Alexander et al., 2000; Delettre et al., 2000). Many of the *OPA1* mutations identified are thought to result in haploinsufficiency or dominant-negative phenotypes (Alexander et al., 2000; Delettre et al., 2000). Furthermore, in patients bearing the R445H mutation in the GTPase domain, sensorineural deafness is also observed (Amati-Bonneau et al., 2005). Examination of mitochondria in monocytes and fibroblasts from ADOA patients shows increased fragmentation and clustering (Delettre et al., 2000; Amati-Bonneau et al., 2005).

Mitochondria have been intricately linked to the process of apoptosis, and there is now mounting evidence to suggest that this involves the participation of mitochondrial morphology components via regulation of fission dynamics (Youle and Karbowski, 2005). For example, Drp1 translocates from the cytosol to foci on mitochondria following the induction of apoptosis (Frank et al., 2001; Sugioka et al., 2004). Furthermore, the proapoptotic Bcl-2 family member Bax is found to co-localise to mitochondrial fission sites with Drp1 and Mfn2 during apoptosis (Karbowski et al., 2002). Similarly, the fission protein endophilin B1 was found to interact with Bax subsequent to induction of apoptosis (Youle and Karbowski, 2005). Over-expression of hFis1 has been found to trigger cytochrome *c* release for the execution of apoptosis, likely due to enhanced fission rates (James et al., 2003). In addition, depletion of endogenous hFis1 inhibits translocation of the pro-apoptotic mediator Bax to the mitochondrial outer membrane and prevents cytochrome *c* release (Sugioka et al., 2004). Whereas depletion of Drp1 had no effect on Bax translocation, cytochrome *c* release was still prevented (Lee et al., 2004). This suggests that the mediators of mitochondrial fission can act to regulate the cellular apoptotic pathway through their regulation of mitochondrial morphology. In addition, recent reports suggest an important role for Ca^{2+} in this regulation, since the apoptotic pathway that involves Ca^{2+} transmission from the endoplasmic reticulum to mitochondria involves recruitment of Drp1 to mitochondria (Breckenridge et al., 2003; Germain et al., 2005).

A recent report indicates that OPA1 is released during apoptosis, along with cytochrome *c*, which likely triggers cristae rearrangements and arrests fusion (Arnoult et al., 2005). Alterations of cristae folds is thought to lead to the further release of previously sequestered protein pools of cytochrome *c*, thereby promoting the rapid exe-

cution of apoptosis (Arnoult et al., 2005; Scorrano, 2005). It is tempting to speculate that the effects of *OPA1* mutations observed in ADOA patients mimic models in which Opa1 depletion leads to increased sensitivity to apoptotic stimuli, providing an explanation of retinal cell dysfunction.

The requirement for mitochondrial fission events and their mechanisms during apoptosis remains to be confirmed. This process may in part rely on remodelling of the mitochondrial cristae, thus releasing sequestered stores of Ca^{2+} and further committing the cell to apoptosis (Scorrano et al., 2002). However, fission of mitochondria does not always contribute to apoptosis. Upon induction of Ca^{2+}-mediated apoptosis, Drp1-induced fission of mitochondria appears to be protective, as it dissipates the transmission of the signal (Szabadkai et al., 2004). The fact that mitochondrial fission events are constantly taking place in healthy cells to regulate mitochondrial morphology indicates that additional stimuli or protein factors must induce changes in the fission mediators to facilitate cytochrome *c* release (Youle and Karbowski, 2005). Such factors or stimuli have yet to be identified.

Conclusion

The past 5 years have seen rapid developments in this area of research, leading to the recognition that control of mitochondrial morphology is essential to the health of the cell. Defects in the distribution and shaping of mitochondria have the potential to cause localised energy and calcium imbalances, resulting in cellular malfunction and death. The characterisation of a number of proteins involved in these processes has also revealed that their defects are linked to a number of diseases. Further development of *in vitro* biochemical assays for both mitochondrial fission and fusion should allow for a greater understanding of the mechanisms involved in controlling mitochondrial shape. In particular, we have little knowledge of the process by which the inner and outer membranes are kept from mixing during both fission and fusion. Further understanding will presumably come through the identification and analysis of additional components involved in regulating morphology, as well as the development of new techniques to study the interplay between membranes and the proteins that shape them.

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