Functions and dysfunctions of mitochondrial dynamics

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Abstract | Recent findings have sparked renewed appreciation for the remarkably dynamic nature of mitochondria. These organelles constantly fuse and divide, and are actively transported to specific subcellular locations. These dynamic processes are essential for mammalian development, and defects lead to neurodegenerative disease. But what are the molecular mechanisms that control mitochondrial dynamics, and why are they important for mitochondrial function? We review these issues and explore how defects in mitochondrial dynamics might cause neuronal disease.

Cristae
Invaginations of the mitochondrial inner membrane.

Nebenkern structure
A cytosolic structure, found in some insect spermatids, that is formed by the fusion of mitochondria.

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In the 1950s, seminal electron microscopy studies led to the canonical view of mitochondria as bean-shaped organelles. These studies revealed the ultrastructural hallmarks of mitochondria, which include double lipid membranes and unusual inner membrane folds termed cristae. Recent studies have led to renewed appreciation for the fact that the mitochondrial structure is highly dynamic[1,2]. Mitochondria have drastically different morphologies depending on the cell type and, even in the same cell, mitochondria can take on a range of morphologies, from small spheres or short rods to long tubules. In fibroblasts, for example, mitochondria visualized with fluorescent proteins or specific dyes typically form tubules with diameters of ~0.5 µm, but their lengths can range from 1–10 µm or more.

Even more remarkably, imaging studies in live cells indicate that mitochondria constantly move and undergo structural transitions. Mitochondrial tubules move with their long axes aligned along cytoskeletal tracks[3]. Individual mitochondria can encounter each other during these movements and undergo fusion, resulting in the merging of the double membranes and the mixing of both lipid membranes and intramitochondrial content (BOX 1). Conversely, an individual mitochondrion can divide by fission to yield two or more shorter mitochondria.

What are the molecular mechanisms that underlie these unusual behaviours, and do they have consequences for mitochondrial function and cell physiology? In this Review, we discuss the dynamic nature of mitochondria and summarize the mechanisms that drive mitochondrial fusion and fission. In addition, we discuss recent insights into how these processes affect the function of mitochondria. As well as controlling the shape of mitochondria, fusion and fission are crucial for maintaining the functional properties of the mitochondrial population, including its respiratory capacity. Moreover, mitochondrial dynamics has key roles in mammalian development, several neurodegenerative diseases and apoptosis.

Mitochondria as dynamic organelles

By several criteria, mitochondria are dynamic organelles. First, the shape and size of mitochondria are highly variable and are controlled by fusion and fission. Second, mitochondria are actively transported in cells and they can have defined subcellular distributions. Finally, the internal structure of mitochondria can change in response to their physiological state.

Dynamic shape. The length, shape, size and number of mitochondria are controlled by fusion and fission (FIG. 1a). At steady state, the frequencies of fusion and fission events are balanced[4] to maintain the overall morphology of the mitochondrial population. When this balance is experimentally perturbed, dramatic transitions in mitochondrial shape can occur. Genetic studies in yeast and mammals indicate that cells with a high fusion-to-fission ratio have few mitochondria, and that these mitochondria are long and highly interconnected[5–8] (FIG. 2). Conversely, cells with a low fusion-to-fission ratio have numerous mitochondria that are small spheres or short rods — these are often referred to as 'fragmented mitochondria'. *In vivo*, such changes in dynamics control mitochondrial morphology during development. For example, during *Drosophila melanogaster* spermatogenesis, many mitochondria synchronously fuse to form the Nebenkern structure, which is required for sperm motility[9].

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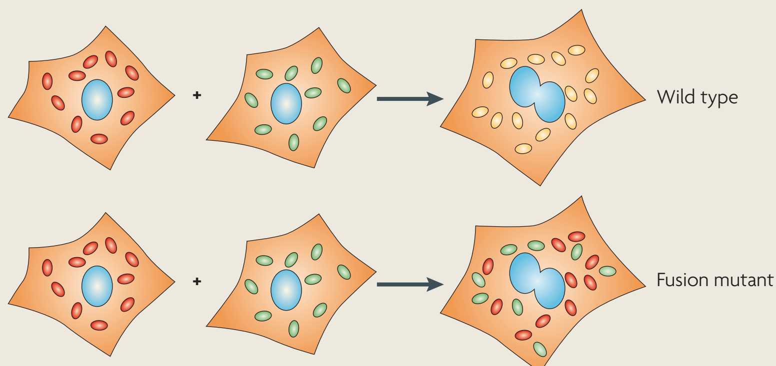
Box 1 | **What happens to mitochondrial components after fusion?**



In time-lapse movies of labelled mitochondria in living cells, mitochondria are observed to undergo cycles of fusion and fission. With each fusion event, two mitochondria are merged into one. Intuitively, true fusion would be expected to involve outer membrane and inner membrane fusion, which would also result in mixing of the matrix contents. Indeed, these expectations have been experimentally confirmed. Apparent fusion events that have been visualized in cells can be confirmed by a cell-hybrid mitochondrial fusion assay[6,32] (see figure). In this assay, mitochondria in two distinct cell lines are differentially labelled with mitochondrially targeted green fluorescent protein (GFP) and DsRed. The cell lines are co-plated onto cover slips and exposed briefly to polyethylene glycol, a chemical that induces adjacent cells to fuse into cell hybrids. After a recovery period, the cell hybrids are examined for mitochondrial fusion. In cell hybrids from normal cells, mitochondrial fusion results in mitochondria that carry both GFP and DsRed (see figure, top). Cells that are defective for mitochondrial fusion form cell hybrids with distinct red and green mitochondria (see figure, bottom). A conceptually similar assay can be performed with yeast cells by allowing labelled yeast strains to mate and form zygotes[4]. By using matrix-targeted fluorophores, these assays show that mitochondrial fusion results in mixing of the matrix contents. Moreover, by using mitochondrial markers that are localized to the outer or inner membranes, fusion of the individual membranes can be experimentally demonstrated; under normal conditions, outer and inner membrane fusion appear to be closely synchronized.

An important question is what happens to mitochondrial DNA (mtDNA) after fusion. Each mitochondrion contains multiple copies of the mtDNA genome that are organized into one or more nucleoids. After fusion, these nucleoids appear to be motile and can potentially interact with each other[73]. In mammalian cells, mtDNA recombination has been documented, but its extent and importance is unclear.

Dynamic subcellular distribution. Mitochondrial transport is required to distribute mitochondria throughout the cell (FIG. 1b). In most cells, mitochondria are highly motile and travel along cytoskeletal tracks. Mitochondrial transport depends on the actin cytoskeleton in budding yeast[10] and on both actin and microtubules in mammalian cells[3,11,12]. Depending on the cellular context, these transport processes can ensure proper inheritance of mitochondria or can recruit mitochondria to active regions of the cell. For example, in budding yeast, mitochondria are transported into and retained in the developing bud to ensure mitochondrial inheritance to the daughter cell[10].

This regulation of mitochondrial distribution is particularly evident in neurons. Quantitative measurements of neuronal mitochondrial transport have reported rates ranging from 0.4 μm min^{-1} (REF. 13) to 0.1–1 μm sec^{-1} (REFS 11,14,15). Such directed movements are not continuous; rather, they are saltatory, with pauses often followed by a reversal of direction. These patterns might reflect the attachment and detachment of cytoskeletal motors. Although these movements can appear chaotic, several lines of evidence from neuronal studies suggest that mitochondrial transport is regulated. First, mitochondria are recruited to regions with high energy demands, including active growth cones, presynaptic sites and postsynaptic sites[13,16,17]. Such recruitment is regulated by neuronal activation, further arguing that the recruitment of mitochondria is responsive to the local metabolic state. Second, neuronal mitochondria pause most often at sites that lack other mitochondria, resulting in a well-spaced axonal mitochondrial distribution[14]. Third, studies with the membrane-potential indicator dye JC-1 suggest that mitochondria with high membrane potential preferentially migrate in the anterograde direction, whereas mitochondria with low membrane potential move in the retrograde direction[14]. These migration patterns suggest that active mitochondria are recruited to distal regions with high energy requirements, whereas impaired mitochondria are returned to the cell soma, perhaps for destruction or repair. Finally, mitochondrial transport along axons responds to local concentrations of nerve growth factor (NGF), suggesting that specific signalling pathways control mitochondrial recruitment and retention[16,18].

Dynamic internal structure. In addition to changes in the overall shape of mitochondria, the internal structures of mitochondria are also dynamic. Three-dimensional tomography of cryopreserved samples has provided new views of inner membrane organization and plasticity[19]. The inner membrane can be divided into distinct regions: the inner boundary membrane, the cristae membrane and the cristae junctions (FIG. 1c). The inner boundary membrane comprises the regions in which the inner membrane is in close proximity to the outer membrane. These regions are probably important for protein import and might be the sites of coupled outer and inner membrane fusion. The cristae junctions are narrow 'neck' regions that separate the inner boundary membrane from the involuted cristae membrane. Cytochrome *c*, an intermembrane-space protein, is enriched in the space that is encased by cristae membranes, and the regulated opening of cristae junctions might be important for its relocalization during apoptosis[20].

These regions of the mitochondrial inner membrane are not only morphologically distinct but also appear to constitute separate functional domains. Proteins that are involved in the translocation of proteins through the inner membrane, such as the TIM23 complex, are enriched in the inner boundary membrane, whereas proteins that are involved in oxidative phosphorylation are enriched in the cristae membranes[21–23]. In addition, the structure of mitochondrial membranes is linked to the metabolic state of mitochondria (FIG. 1c). Purified mitochondria placed in low ADP conditions have limited respiration and have an 'orthodox' morphology, characterized by narrow cristae and few cristae junctions per cristae compartment. Under high ADP and substrate conditions, isolated mitochondria have high respiratory activity and a 'condensed' morphology, characterized by larger cristae and several cristae junctions per cristae compartment[19]. It is unknown how inner membranes convert between these states, but inner membrane fusion

Anterograde
The direction from the cell body towards the periphery.

Retrograde
The direction from peripheral regions towards the cell body.

Oxidative phosphorylation
A biochemical pathway for ATP production that results in oxygen consumption and is localized to the mitochondrial cristae.

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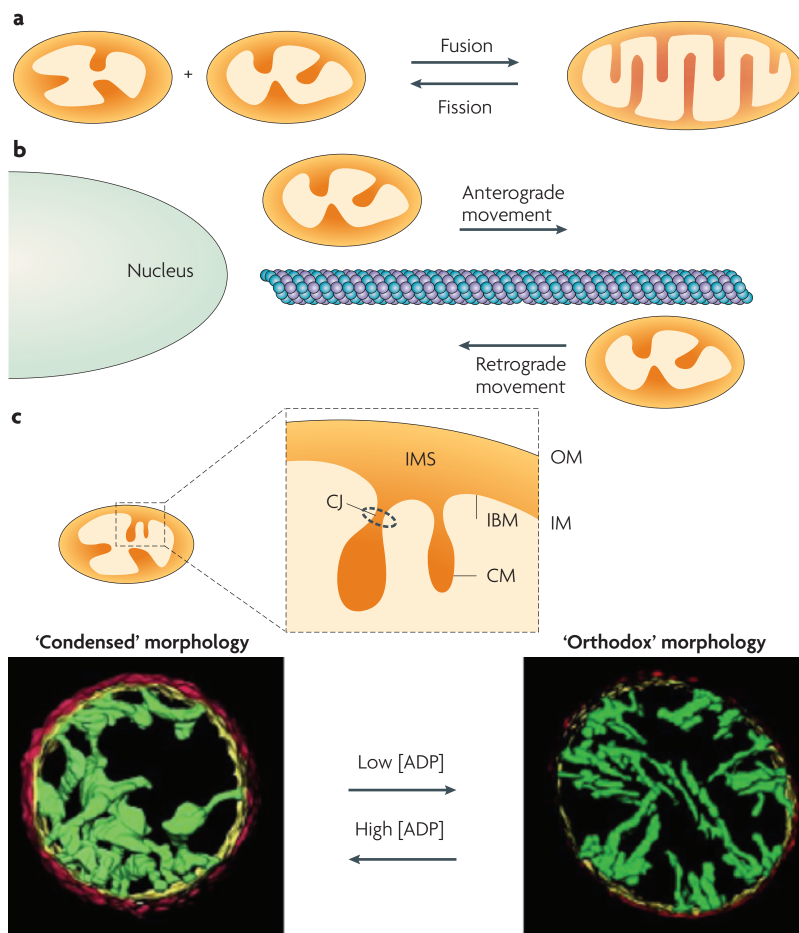

Figure 1 | **Mitochondria as dynamic organelles. a** | Mitochondrial fusion and fission control mitochondrial number and size. With fusion, two mitochondria become a single larger mitochondrion with continuous outer and inner membranes. Conversely, a single mitochondrion can divide into two distinct mitochondria by fission. **b** | In mammalian systems, mitochondria are distributed throughout the cytoplasm by active transport along microtubules and actin filaments. Distinct molecular motors transport the mitochondria in anterograde or retrograde directions. **c** | Inner membrane dynamics. The diagram indicates the different regions of the inner membrane. The bottom panels show electron microscopy (EM) tomograms of two mitochondria under different metabolic conditions (red, outer membrane; yellow, inner boundary membrane; green, cristae membrane). Cristae organization can vary widely, often in response to the bioenergetic state of the cell: an 'orthodox' cristae morphology, with narrow cristae and few cristae junctions per cristae compartment, is found in low ADP conditions, whereas a 'condensed' morphology, with larger cristae and several junctions per cristae compartment, is found in high ADP conditions[19]. EM images reproduced with permission from REF. 19 © (2006) Elsevier. CJ, cristae junction; CM, cristae membrane; IBM, inner boundary membrane; IM, inner membrane; IMS, intermembrane space; OM, outer membrane.

Coiled coil
A structural motif that is formed by polypeptide sequences that contain hydrophobic heptad repeats.

Dynamin
A large GTPase that is thought to mediate vesicle scission during endocytosis.

and fission might be involved[19]. Taken together, these observations indicate that inner membrane morphology is intimately related to bioenergetics, although the causal relationship remains unclear.

Mediators of fusion and fission

Molecular analysis of mitochondrial morphology began with the discovery in 1997 of the *D. melanogaster* fusion factor fuzzy onions (FZO), a mitochondrial outer membrane GTPase that is required for the fusion of mitochondria during spermatogenesis[9]. FZO is the founding member of the mitofusin family of GTPases. The yeast orthologue, Fzo1, has a conserved role in mitochondrial fusion[24], and genetic screens in yeast have identified additional modulators of mitochondrial fusion and fission[2,25] (FIG. 3b). The core machineries that mediate mitochondrial fusion and fission are best understood in yeast. Several of these components have functionally conserved mammalian homologues. More comprehensive discussions of the molecular mechanisms of mitochondrial fusion and fission have been presented in recent reviews (for example, see REF. 1).

Mitochondrial fusion. In yeast, the core mitochondrial fusion machinery consists of two GTPases: Fzo1 and Mgm1 (FIG. 3). Fzo1 is located on the mitochondrial outer membrane and is essential for fusion of the outer membranes[24,26]. The mammalian homologues of Fzo1 are the mitofusins MFN1 and MFN2. These two related proteins form homo-oligomeric and hetero-oligomeric complexes that are essential for fusion[6,27,28]. Mitofusins are required on adjacent mitochondria during the fusion process, implying that they form complexes in *trans* between apposing mitochondria[26,29]. A heptad repeat region of MFN1 has been shown to form an antiparallel coiled coil that is probably involved in tethering mitochondria during fusion[29].

Mgm1 is a dynamin-related protein that is essential for fusion of the mitochondrial inner membranes in yeast[30], a function that is consistent with its localization to the intermembrane space and its association with the inner membrane. The mammalian orthologue OPA1 is also essential for mitochondrial fusion[28,31]. In yeast, the outer membrane protein Ugo1 physically links Fzo1 and Mgm1, but no mammalian orthologue has yet been discovered[2].

The membrane potential across the mitochondrial inner membrane is maintained by the electron transport chain and is essential for mitochondrial fusion[26,32]. Ionophores that dissipate the mitochondrial membrane potential cause mitochondrial fragmentation, owing to an inhibition of mitochondrial fusion[32,33]. In an *in vitro* fusion assay, both the proton and the electrical gradient components of the membrane potential are important[26]. The mechanistic link between membrane potential and fusion remains to be resolved, but one factor appears to be the dependence of post-translational processing of OPA1 on the membrane potential[34].

Recent work has also identified mitochondrial lipids as important factors in fusion. Mitochondrial morphology screens in yeast identified members of the ergosterol synthesis pathway as being required for normal mitochondrial morphology[35,36]. Recently, mitochondrial phospholipase D has been identified as a protein that is important for mitochondrial fusion[37]. This mitochondrial outer membrane enzyme hydrolyses cardiolipin to generate phosphatidic acid. Interestingly, ergosterol has been associated with yeast vacuole fusion[38], and phosphatidic acid is thought to play a part in generating the membrane curvature that is required for SNARE-mediated fusion[39]. Thus, specific lipids might have similar roles in distinct types of membrane fusion.

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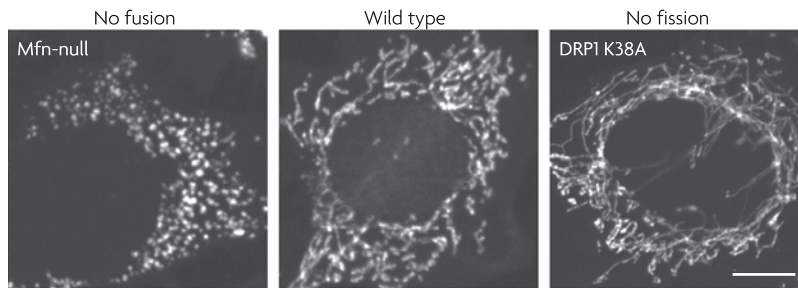

Figure 2 | **Mitochondrial fusion and fission regulate morphology.** Mitochondrial length, size and connectivity are determined by the relative rates of mitochondrial fusion and fission. In wild-type cells (shown in the central panel), mitochondria form tubules of variable length. In the absence of mitochondrial fusion (for example, in mitofusin (Mfn)-null cells (shown in the left panel), which lack MFN1 and MFN2), unopposed fission results in a population of mitochondria that are all fragmented. Conversely, decreased fission relative to fusion (for example, in DRP1 K38A cells (shown in the right panel), which have a dominant-negative form of dynamin-related protein-1 (DRP1)) results in elongated and highly interconnected mitochondria. Scale bar represents 10 μm.

Mitochondrial membrane potential
The electrochemical gradient that exists across the mitochondrial inner membrane.

Ergosterol
A steroid compound that is a component of yeast cell membranes and which might have a role similar to that of cholesterol in mammalian cell membranes.

SNARE
(soluble *N*-ethylmaleimide-sensitive fusion protein (NSF) attachment protein (SNAP) receptor). A highly α-helical protein that mediates the specific fusion of vesicles with target membranes.

F-box protein
A protein containing an F-box motif, a small domain that is used for protein interactions. The best-characterized F-box proteins are components of an E3 ubiquitin ligase, and help in ubiquitin-dependent protein degradation by recognizing specific substrates.

β-barrel protein
A protein composed of a β-sheet that is rolled up into a cylinder. One such mitochondrial β-barrel protein is VDAC (voltage-dependent anion channel), which forms a pore in the outer membrane.

Kinesin
A microtubule-based molecular motor protein that is most often directed towards the plus end of microtubules.

Mitochondrial fission. Mitochondrial fission requires the recruitment of a dynamin-related protein (Dnm1 in yeast and DRP1 in mammals) from the cytosol (FIG. 4). Both Dnm1 and DRP1 assemble into punctate spots on mitochondrial tubules, and a subset of these complexes lead to productive fission events[5,7,8]. By analogy with the classical function of dynamin in endocytosis, Dnm1 and DRP1 are thought to assemble into rings and spirals that encircle and constrict the mitochondrial tubule during fission[25]. Consistent with this model, purified Dnm1 can indeed form helical rings and spirals *in vitro*, with dimensions that are similar to those of constricted mitochondria[40]. Moreover, Dnm1 assembly is required for fission activity[41].

The recruitment of Dnm1 to yeast mitochondrial fission sites involves three other components. One of these is Fis1, a mitochondrial integral outer membrane protein that is essential for fission[42–44]. Fis1 binds indirectly to Dnm1 through one of two molecular adaptors, Mdv1 or Caf4 (REF. 45) (FIG. 4b). Either Mdv1 or Caf4 is sufficient to allow the Fis1-dependent recruitment of Dnm1, although Mdv1 has a more important role in mediating fission. FIS1, the mammalian homologue of Fis1, is also essential for mitochondrial fission[46], but no homologues of Mdv1 or Caf4 are currently known. FIS1 and DRP1 are also required for the fission of peroxisomes[47,48].

Other regulators of dynamics

Mitochondrial fusion and fission activities are probably coordinated with cellular physiology. In yeast, the steady-state levels of Fzo1 are controlled by the F-box protein Mdm30, which negatively regulates Fzo1 levels in a proteasome-independent manner[49,50]. In mammalian cells, post-translational modification of DRP1 regulates its function in mitochondrial fission. The mitochondrial E3 ubiquitin ligase MARCH5 is essential for mitochondrial fission[51]. This requirement is probably related to the ability of MARCH5 to promote DRP1 ubiquitylation and to associate physically with ubiquitylated DRP1 (REFS 52,53). Furthermore, during apoptosis, sumoylation of DRP1 is activated in a BAX- and/or BAK-dependent manner[54]. This modification of DRP1 might affect its association with mitochondrial membranes. Mitochondrial fission is also regulated by the cell cycle. For example, mitochondria in HeLa cells are usually tubular, but they become more fragmented during mitosis, a phenomenon that might facilitate the partitioning of mitochondria to daughter cells during cytokinesis. This regulated fragmentation of mitochondria is due to increased mitochondrial fission, and phosphorylation of DRP1 during mitosis has been implicated[55].

In addition to the genes that encode core fusion and fission components, other genes can affect mitochondrial morphology. Large-scale visual screens for aberrant mitochondrial morphology in mutant yeast have yielded numerous genes of interest and provided general insights into the control of mitochondrial morphology[35,36]. These screens suggest that several cellular pathways influence mitochondrial morphology and inheritance, including ergosterol biosynthesis, mitochondrial protein import, actin dynamics, vesicular fusion and ubiquitin-mediated protein degradation. The close interplay between mitochondrial protein import and morphology has been emphasized by the recent finding that the mitochondrial morphology genes *MMM1*, *MDM10* and *MDM12* have a direct role in the assembly of β-barrel proteins in the outer mitochondrial membrane[56].

Proteins that are required for mitochondrial transport. Energy-dependent molecular motors transport mitochondria along cytoskeletal filaments. Along microtubules, multiple kinesin family members and cytoplasmic dynein have been implicated in anterograde and retrograde mitochondrial transport, respectively[3]. Recent work has clarified the linkage between mitochondria and kinesin-1. Genetic screens in *D. melanogaster* identified *milton* and *Miro*, both of which are required for anterograde mitochondrial transport in neurons[57,58]. Milton interacts directly with kinesin and Miro, which is a mitochondrial outer membrane protein that has GTPase and Ca^{2+}-binding EF-hand domains[59]. In yeast, disruption of the Miro orthologue Gem1 results in abnormalities in mitochondrial morphology and poor respiratory activity[60]. Both GTP-binding and Ca^{2+}-binding motifs are essential for Gem1 function, which appears not to be involved in fusion or fission. Depending on the cell type, mitochondria can also travel along actin filaments under the control of myosin motors[3].

Proteins that mediate inner membrane morphology. Studies of mitochondrial inner membrane structure are complicated by the intimate link between mitochondrial bioenergetics and cristae structure. As a result, disruption of the proteins that are important for bioenergetics can lead to a secondary effect on inner membrane structure. Nevertheless, several proteins probably have a specific role in controlling cristae structure. In addition to their roles in mitochondrial fusion, Mgm1 and OPA1 are important for cristae structure. Loss of Mgm1 in yeast or knockdown of OPA1 in mammalian cells results in disorganized inner membrane structures[30,61–64]. In both cases, homo-oligomeric interactions are involved[30,64].

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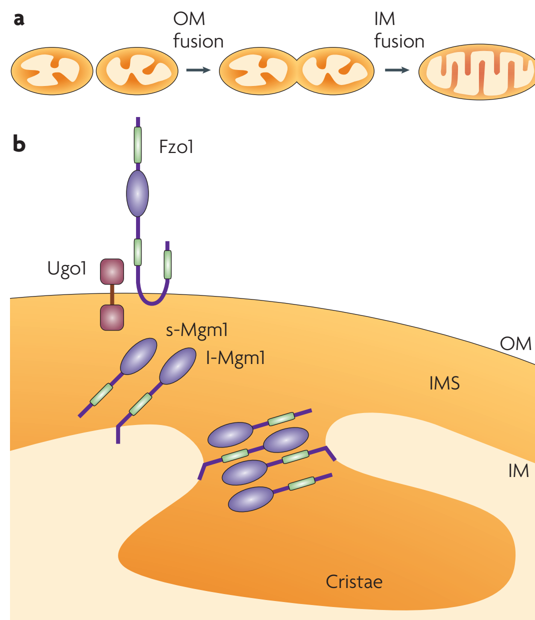

Figure 3 | **Mitochondrial fusion. a** | Mitochondrial fusion consists of outer membrane (OM) fusion followed by inner membrane (IM) fusion. Normally these events occur coordinately. **b** | The dynamin-related proteins Fzo1 and Mgm1 are key molecules in the yeast mitochondrial fusion machinery. Fzo1 is an integral outer membrane protein with GTPase and heptad repeat domains that face the cytoplasm. All of the domains are required for the fusion activity of Fzo1. Mgm1 is present on the inner membrane, facing the intermembrane space (IMS), and is proteolytically processed by a rhomboid protease. Both long (l-Mgm1) and short (s-Mgm1) forms are required for mitochondrial fusion. In addition to inner membrane fusion, Mgm1 is required for the maintenance of cristae structures. Ugo1 binds to both Fzo1 and Mgm1 and probably coordinates their function. All components are encoded by nuclear DNA. The mitofusin proteins MFN1 and MFN2 are the mammalian homologues of Fzo1; OPA1 is the mammalian homologue of Mgm1. No mammalian homologue of Ugo1 has been identified so far.

Dynein
A microtubule-based molecular motor that is directed towards the minus end of microtubules.

EF-hand domain
A helix-loop-helix protein motif that can bind a Ca^{2+} ion.

Mitochondrial F_1F_0 ATP synthase
A large, multisubunit enzyme embedded in the mitochondrial cristae that uses the proton gradient across the inner membrane to synthesize ATP.

Mitochondrial DNA
(mtDNA). A circular genome (~16 kb in mammals) located in the mitochondrial matrix that encodes 13 polypeptides of the electron transport chain, 22 tRNAs and 2 rRNAs.

Nucleoid
A compacted mass of DNA. Mitochondrial DNA is organized into nucleoids, each consisting of several mitochondrial genomes.

Mitochondrial F_1F_0ATP synthase, a rotary enzyme embedded in the inner membrane that couples proton pumping to ATP synthesis, is essential for normal cristae structure[65]. This role in inner membrane structure involves a dimeric form of ATP synthase that contains the additional subunits e and g. As visualized by electron microscopy, the ATP synthase dimer has a dimeric interface with a sharp angle that could distort the local lipid membrane. This distortion might contribute to the high membrane curvature that characterizes cristae tubules[66,67]. Mgm1 is required for the oligomerization of ATP synthase, providing a link between these two modulators of cristae structure[63].

Additional proteins modulate inner membrane dynamics. In yeast, Mdm33 is required for normal mitochondrial morphology and its overexpression leads to septation and vesiculation of the inner membranes[68]. Because of these phenotypes, Mdm33 has been suggested to have a role in inner membrane fission. Knockdown of mitofilin in mammalian cells causes dramatic abnormalities of the cristae, resulting in the formation of complex layers of inner membrane[69]. Depletion of Mmm1, Mdm31 and Mdm32 — yeast proteins implicated in mitochondrial (mt) DNA maintenance — also cause aberrant cristae morphologies[70,71].

Biological functions of mitochondrial dynamics

Why do mitochondria continually fuse and divide? Recent studies show that these processes have important consequences for the morphology, function and distribution of mitochondria. First, fusion and fission control the shape, length and number of mitochondria. The balance between these opposing processes regulates mitochondrial morphology. Second, fusion and fission allow mitochondria to exchange lipid membranes and intramitochondrial content. Such exchange is crucial for maintaining the health of a mitochondrial population. Third, the shape of mitochondria affects the ability of cells to distribute their mitochondria to specific subcellular locations. This function is especially important in highly polarized cells, such as neurons. Finally, mitochondrial fission facilitates apoptosis by regulating the release of intermembrane-space proteins into the cytosol. As a result of these cellular functions, mitochondrial dynamics has consequences for development, disease and apoptosis.

Maintaining a healthy mitochondrial population. Mitochondrial fusion is required to maintain a functional mitochondrial population in the cell. Fibroblasts that lack both MFN1 and MFN2 have reduced respiratory capacity, and individual mitochondria show great heterogeneity in shape and membrane potential[28]. Cells that lack OPA1 show similar defects, with an even greater reduction in respiratory capacity.

How does fusion protect mitochondrial function? It is probable that a primary function of mitochondrial fusion is to enable the exchange of contents between mitochondria (BOX 1). As a result, mitochondria should not be considered autonomous organelles; instead, the hundreds of mitochondria in a typical cell exist as a population of organelles that cooperate with each other through fusion and fission. The heterogeneous properties of mitochondria in fusion-deficient cells are consistent with this model[28]. In normal cells, a few mitochondria might be non-functional owing to the loss of essential components. However, this dysfunction is transient because mitochondrial fusion provides a pathway for these defective mitochondria to regain essential components (FIG. 5a).

An essential component of mitochondrial function is mitochondrial DNA (mtDNA), which is organized into compact particles termed nucleoids. The mtDNA genome encodes essential subunits of the respiratory complexes I, III and IV, and is therefore essential for oxidative phosphorylation. When mitochondrial fusion is abolished, a large fraction of the mitochondrial population loses mtDNA nucleoids[72]. During mitochondrial division in normal cells, most daughter mitochondria inherit at least one mtDNA nucleoid[73]. However, in cases where a

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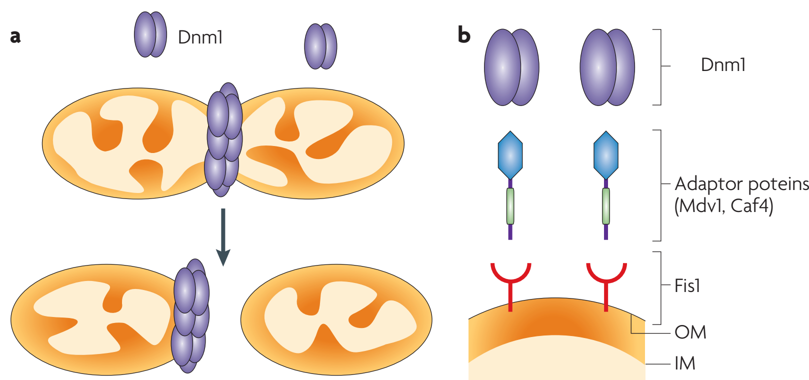

Figure 4 | **Mitochondrial fission. a** | In yeast, mitochondrial fission is mediated by the dynamin-related protein Dnm1. Cytoplasmic Dnm1 localizes to the mitochondrial outer membrane (OM), where it oligomerizes into a ring structure that constricts and severs the mitochondrion. In this model, Dnm1 functions in an analogous manner to the way dynamin functions in endocytosis. **b** | The localization of Dnm1 on the mitochondrial outer membrane is mediated by Fis1 and the adaptor proteins Mdv1 and Caf4. Fis1 is an integral outer membrane protein that interacts with the N termini of Mdv1 and Caf4. Both Mdv1 and Caf4 have C-terminal WD-40 repeats that bind Dnm1. Fis1 and Dnm1 have mammalian homologues (FIS1 and DRP1, respectively), but no Mdv1 or Caf4 homologues have been identified so far. IM, inner membrane.

Chemotaxis
The directed movement of cells in response to a chemical stimulus.

daughter fails to inherit a nucleoid, mitochondrial fusion would enable restoration of mtDNA. In fusion-deficient cells, the lack of content exchange prevents restoration of mtDNA nucleoids and probably accounts for the heterogeneity in membrane potential and the reduced respiratory capacity. It should be noted that fusion-deficient cells still maintain significant numbers of mtDNA nucleoids; however, due to ongoing mitochondrial fission, these nucleoids are encased by a small mitochondrial mass, and therefore the functional mitochondrial mass (at least in terms of bioenergetics) in such cells is greatly reduced (FIG. 5b). In addition to mtDNA, it is also possible that other components, such as substrates, metabolites or specific lipids, can be restored in defective mitochondria by fusion. Further studies will determine whether content exchange is the primary function of mitochondrial fusion. The importance of mitochondrial fusion in development and disease might be a consequence of this function.

Essential developmental functions. Perturbations in mitochondrial dynamics result in specific developmental defects. Mice that lack either MFN1, MFN2 or OPA1 fail to survive past mid-gestation[6,74,75]. MFN2 has a highly specific function in the development of the trophoblast giant cell layer of the placenta[6]. Likewise, MFN1 appears to have an essential placental function[72].

Mitochondrial fission is also an essential process. Worms that are deficient in mitochondrial division die before adulthood[76]. An infant patient with a dominant-negative *DRP1* allele has been reported. This patient died at ~1 month of age and had a wide range of abnormalities, including reduced head growth, increased lactic acid and optic atrophy. Fibroblasts from this patient showed elongated mitochondria and peroxisomes[77]. It is unclear how the developmental defects are related to these organellar shape changes.

Mitochondrial distribution and recruitment in neurons. Given the importance of mitochondrial dynamics in maintaining bioenergetics, these dynamics are probably a ubiquitous phenomenon that is important for all cells. However, certain cells, particularly neurons, seem to be especially dependent on its proper control. This dependence of neurons probably stems from their high energy demands and the special importance of proper mitochondrial distribution: mitochondria are concentrated in several neuronal regions, including pre- and postsynaptic sites[13,17]. To achieve this non-uniform distribution, neurons rely heavily on active transport to recruit mitochondria and other organelles to nerve terminals[3].

The proper localization of mitochondria to axon terminals depends on mitochondrial dynamics. Neurons that lack Milton, Miro or DRP1 show defective mitochondrial transport and have sparse mitochondria at axon terminals. Such distribution defects lead to reduced capacity for synaptic transmission[57,58,78]. It seems likely that mitochondria that are localized to synapses are primarily required to drive ATP-dependent processes. The synapses of neurons that express mutant DRP1 show defective mobilization of the reserve vesicle pool (an ATP-dependent process), and the defects in synaptic transmission can be rescued by experimentally filling synapses with ATP. In addition, synapse-localized mitochondria help to regulate Ca^{2+} homeostasis, although this function appears to be crucial only during intense synaptic activity. Synapses that lack Miro or DRP1 have elevated resting Ca^{2+} levels, but normal Ca^{2+} dynamics are maintained except under sustained nerve stimulation[57,78].

Both mitochondrial fusion and fission affect the mitochondrial distribution in dendrites. In hippocampal neurons, mitochondria accumulate at dendritic spines following neuronal stimulation[13]. Inhibition of mitochondrial fission causes elongation of the mitochondria and decreases the abundance of dendritic mitochondria and the density of dendritic spines. Conversely, increased fission facilitates the mobilization of dendritic mitochondria and leads to an increased spine number[13]. In the cerebellum, the distribution of mitochondria in the dendritic processes of Purkinje neurons is highly dependent on mitochondrial fusion[72] (see below).

Lymphocyte chemotaxis. Mitochondrial dynamics appears to be important for proper mitochondrial redistribution in lymphocytes during chemotaxis[79]. Mitochondria are concentrated in the trailing edge in lymphocyte cell lines that migrate in response to chemical attractants. Modulation of mitochondrial fusion or fission affects both mitochondrial redistribution and cell migration. Fragmentation enhances mitochondrial redistribution and cell migration, whereas conditions that promote fusion have the opposite effect. Therefore, as in neurons, mitochondrial shape in lymphocytes can affect the recruitment of mitochondria to local cellular areas.

Regulation of apoptosis. In apoptosis, several structural changes occur in mitochondria during the early phase of cell death (FIG. 6). The mitochondria become fragmented owing to increased fission activity. At approximately the

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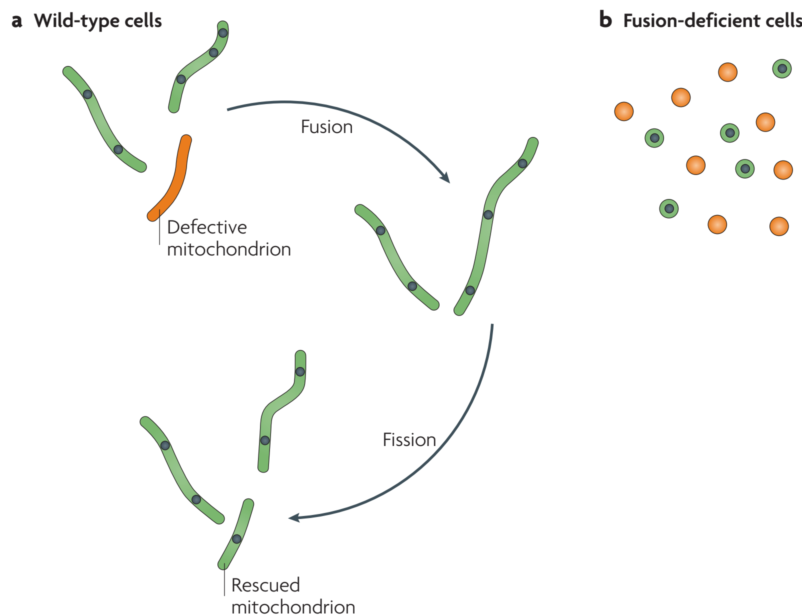

Figure 5 | **Mitochondrial dynamics protects mitochondrial function.** **a** | In wild-type cells, the vast majority of mitochondria are functional (shown in green). In this simplified diagram, one mitochondrion is depicted as non-functional (shown in orange). One of several possible reasons for dysfunction is a lack of mitochondrial DNA (mtDNA) nucleoids (shown as black circles). The dysfunctional mitochondrion can regain its function and mtDNA by fusing with a neighbouring mitochondrion. The fused mitochondrion then undergoes fission, with both daughter mitochondria receiving mtDNA nucleoids. It should be noted that the identities of the daughter mitochondria are distinct from the parental mitochondria, owing to content exchange and the fact that the fission point is typically distinct from the fusion point. **b** | In fusion-deficient cells, mitochondria are fragmented due to ongoing fission in the absence of fusion. Mitochondria that lack mtDNA nucleoids accumulate because there is no pathway for defective mitochondria to regain mtDNA. Fusion-deficient cells can maintain mtDNA nucleoids, but such nucleoids serve a much smaller mitochondrial mass.

same time, mitochondrial outer membrane permeabilization (MOMP) causes the release of contents of the intermembrane space, such as cytochrome *c* and second mitochondria-derived activator of caspase (SMAC)/Diablo, into the cytoplasm. Because cytochrome *c* is preferentially sequestered in cristae compartments, it is thought that the opening of cristae junctions is a vital step in facilitating its efficient release. Once in the cytosol, cytochrome *c* activates a cascade of caspases that propagate and execute the apoptotic programme. These three structural changes — fragmentation, MOMP and cristae remodelling — occur at similar times, but their temporal sequence and causative links are still controversial[80,81].

Mitochondrial fragmentation during apoptosis is associated with dynamic changes in the mitochondrial localization of several proteins, including BAX, BAK, MFN2, endophilin and DRP1 (REF. 81). Inhibition of fission activity blocks mitochondrial fragmentation, reduces cytochrome *c* release and can reduce or delay cell death depending on the experimental system[46,82,83]. In *Caenorhabditis elegans* and *D. melanogaster*, disruption of DRP1 reduces the number of cell deaths[84–86]. In multiple systems, it seems that fission is important for rapid and efficient cell death, although apoptosis can occur in the absence of mitochondrial fission. An important issue to resolve in future studies is how fission is related to the permeabilization of mitochondria.

Surprisingly, the apoptotic proteins BAX and BAK, which have well-established pro-apoptotic roles in mitochondrial membrane permeabilization, also appear to regulate mitochondrial morphology. BAX and BAK double-knockout cells have fragmented mitochondria due to reduced mitochondrial fusion[87], although the extent of this effect depends on the experimental system[88]. Little is known about how BAX and BAK mediate their effects on mitochondrial morphology, but BAX influences MFN2 distribution on the mitochondrial outer membrane[87] and BAK associates with MFN1 and MFN2 (REF. 88).

In conjunction with MOMP, remodelling of the cristae membranes is required for the rapid and efficient release of cytochrome *c*[20,89]. Most cytochrome *c* is localized to cristae compartments[20]; OPA1 appears to regulate the diameter of cristae junctions and therefore regulates cytochrome *c* release[64,90]. Overexpression of OPA1 blocks cytochrome *c* release following the induction of apoptosis by maintaining narrow cristae junctions[64]. DRP1 has also been proposed to play a part in cristae remodelling during apoptosis[91].

Role in human disease

Several human diseases are caused by mutations in genes that are essential for mitochondrial dynamics (TABLE 1). Each of these diseases causes degeneration of specific nerves, reinforcing the notion that neurons are particularly prone to defects in mitochondrial dynamics.

OPA1 and autosomal dominant optic atrophy. Heterozygous mutations in OPA1 cause autosomal dominant optic atrophy (ADOA), the most common heritable form of optic neuropathy[92,93]. This disease is characterized by the degeneration of retinal ganglion cells, the axons of which form the optic nerve. More than 100 pathogenic OPA1 mutations have been reported, with most occurring in the GTPase domain[94]. Half of the mutants encode a truncated protein owing to a nonsense mutation. A few nonsense mutations abolish nearly the entire coding sequence, suggesting that haploinsufficiency of OPA1 can cause ADOA. It remains possible that other, less severe, truncations might have dominant-negative activity.

How these OPA1 mutations cause the clinical symptoms of ADOA remains to be clarified. Non-neuronal cells from patients with ADOA can have aggregated, fragmented or normal mitochondria[93,95]; however, because data from only a few patients have been reported, it is not clear whether these findings are the norm. In addition, OPA1 mutations have been associated with reduced ATP production and reduced mtDNA content[96,97]. The defects that have been documented in human ADOA diseased tissue are not as severe as those observed in experimental cells in which OPA1 is depleted. Fibroblasts that are deficient for OPA1 have fragmented mitochondria, defects in respiration, aberrant cristae structure and increased susceptibility to apoptosis[28,31,62,98].

Mitochondrial outer membrane permeabilization (MOMP). The opening of pores in the mitochondrial outer membrane — an early event during apoptosis that releases apoptotic factors from the mitochondrial intermembrane space.

Haploinsufficiency A genetic state in diploids in which a single functional copy of a gene is insufficient to maintain a normal phenotype.

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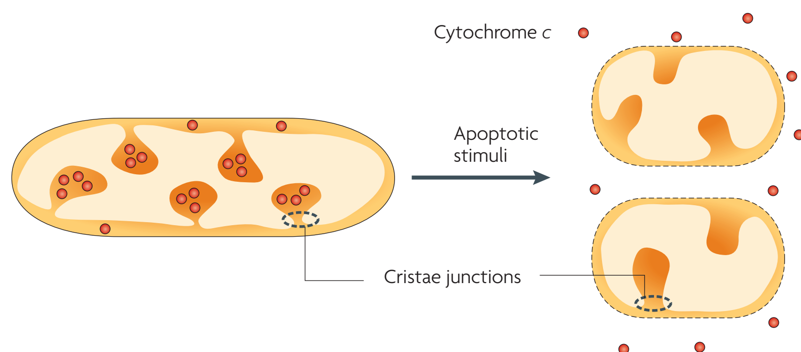

Figure 6 | **Mitochondrial dynamics during apoptosis.** At an early stage of apoptosis, three structural changes occur in mitochondria. Fragmentation takes place as a result of increased fission mediated by dynamin-related protein-1 (DRP1) and the mitochondrial fission-1 protein (FIS1). Mitochondrial outer membrane permeabilization (MOMP; indicated by dashed outlines) is induced by the pro-apoptotic BCL2-family members BAX and BAK. MOMP enables the release of cytochrome *c* (shown as red dots) and other soluble proteins from the intermembrane space. However, release of cytochrome *c* is efficient only if the cristae junctions are widened to allow escape from the cristae compartments. The dynamin-related proteins OPA1 and DRP1 have been implicated in cristae remodelling.

Mouse models of ADOA that contain OPA1 mutations develop the features of ADOA in an age-dependent manner[74,75]. Heterozygous mice show a progressive decline in retinal ganglion cell number and aberrations of axons in the optic nerve. Mice that are homozygous for the OPA1 mutation die at mid-gestation[74,75], which is consistent with an essential requirement for mitochondrial fusion during embryonic development[6].

MFN2 and Charcot-Marie-Tooth 2A. Charcot-Marie-Tooth (CMT) disease, one of the most common hereditary neuropathies, is caused by mutations in at least 30 different genes[99]. Affected individuals have progressive distal motor and sensory impairments that start in the feet and hands as a result of the degeneration of the long peripheral nerves. Depending on the type of CMT, these diseases are caused by either a primary defect in the Schwann cells that myelinate the peripheral nerves or by a defect in the neurons themselves[99]. CMT2A is an axonopathy that is caused by the latter type of defect, and it has been associated with >40 mutations in MFN2. Nearly all of these disease alleles contain missense mutations or short, in-frame deletions[100]. Most mutations cluster in or near the GTPase domain, but some also occur in each of the heptad repeat domains of MFN2. In addition to the loss of peripheral nerve function, a subset of patients with CMT2A have optic atrophy, suggesting that OPA1 and MFN2 mutations can lead to overlapping clinical outcomes[101,102].

Sural nerve
A sensory nerve innervating the calf and foot that is commonly investigated by biopsy for the evaluation of peripheral neuropathies.

Because of the difficulties in studying nerve tissue from patients, the pathogenic mechanisms that lead to peripheral nerve degeneration in CMT2A are not well understood. Only one study has reported ultrastructural defects in mitochondria from the nerves of patients with CMT2A. Mitochondria in the sural nerve of two patients showed structural aberrations in their outer and inner membranes, along with swelling that is suggestive of mitochondrial dysfunction[103]. Aggregation of mitochondria was also observed. Interestingly, CMT2A alleles of *MFN2* (REFS 27,104) cause mitochondrial aggregation and subsequent mitochondrial transport defects in neurons[104]. However, the mitochondrial aggregation phenotype is dependent on significant overexpression[27], and therefore its relevance to disease pathogenesis remains to be clarified.

Several perplexing issues remain to be resolved concerning the molecular genetics of CMT2A. How does mutation of one copy of *MFN2* lead to disease? Why are long peripheral neurons selectively affected, given that MFN2 is a broadly expressed protein? Clues to these issues have come from analysis of CMT2A alleles in mice[27]. Many CMT2A alleles of *Mfn2* are non-functional for fusion when expressed alone. However, the fusion activity of these non-functional alleles can be efficiently complemented by wild-type MFN1 (but not MFN2). This complementation is due to the ability of MFN1 and MFN2 to form hetero-oligomeric complexes that are functional for fusion. In a patient with CMT2A, therefore, cells that express MFN1 are protected from gross loss of fusion activity. By contrast, cells with little or no MFN1 expression suffer a greater relative loss of fusion activity. In part, these properties of the CMT2A alleles might underlie the selective loss of sensory and motor neurons. Consistent with this model, MFN2 seems to be more highly expressed in central and peripheral nervous tissue than MFN1 (S.A.D. and D.C.C., unpublished observations). Even in the peripheral nerves, it appears that mitochondrial fusion defects are only partial because only the longest nerves are affected. Most probably, the extreme dimensions of the long peripheral nerves make them most vulnerable to changes in mitochondrial dynamics.

How might perturbations in mitochondrial fusion lead to neurodegeneration? Clues to the pathogenic mechanisms have come from the finding that mice that lack MFN2 show highly specific degeneration of Purkinje neurons in the cerebellum, resulting in cerebellar ataxia[72]. Purkinje cells are the sole efferent neurons of the cerebellum, and they have exquisitely formed dendritic processes. Both developing and mature Purkinje cells that lose MFN2 fail to support dendritic outgrowth, particularly that of dendritic spines, which are the sites of synaptic connections. In normal Purkinje cells, abundant tubular mitochondria reside in dendritic processes. By contrast, mutant Purkinje cells have fragmented mitochondria that fail to distribute effectively along dendritic

Table 1 | **Disorders associated with mitochondrial perturbations**

Disease	Mitochondrial function	Gene	Description
CMT2A	Fusion	*MFN2*	Autosomal dominant peripheral neuropathy
ADOA	Fusion	*OPA1*	Autosomal dominant optic atrophy (ADOA)
CMT4A	Fission?	*GDAP1*	Autosomal recessive peripheral neuropathy
Unnamed	Fission	*DRP1*	Neonatal lethality

CMT, Charcot-Marie-Tooth; DRP1, dynamin-related protein-1; GDAP1, ganglioside-induced differentiation-associated protein-1; MFN2, mitofusin-2; OPA1, optic atrophy-1.

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processes. In addition, the Purkinje cells show a loss of respiratory activity, probably owing to an accumulation of mitochondria that lack mtDNA nucleoids. Therefore, loss of mitochondrial fusion in Purkinje neurons impairs respiratory activity and mitochondrial localization.

GDAP1 and Charcot-Marie-Tooth 4A. Another form of CMT is associated with defects in mitochondrial dynamics. Ganglioside-induced differentiation-associated protein-1 (GDAP1) is mutated in CMT4A, one of the few recessive forms of CMT disease. CMT4A has both demyelinating and axonal features and, consistent with this mixed clinical presentation, GDAP1 is expressed in both Schwann cells and neurons[105]. GDAP1 is an integral outer membrane protein that probably affects mitochondrial division[105]. Disease alleles either fail to localize to mitochondria or are defective in stimulating mitochondrial fission when overexpressed[105]. If GDAP1 disease alleles disrupt normal mitochondrial fission, they might cause mitochondrial distribution defects similar to those that are induced by the DRP1 mutations discussed above[13,78].

Perspectives

The study of mitochondrial dynamics has undergone great advances in the past few years. It is now clear that mitochondrial dynamics is important for the functional state of mitochondria. By enabling content exchange between mitochondria, fusion and fission prevent the accumulation of defective mitochondria. These opposing processes also control mitochondrial shape, which affects the distribution of mitochondria as well as their participation in apoptosis. As a result, mitochondrial dynamics is particularly important in cells and tissues that have a special dependence on mitochondrial function. Defects in mitochondrial dynamics can manifest in mammalian development, apoptosis and disease. As our knowledge of mitochondrial dynamics increases, we can expect to learn about its involvement in other processes. The link between defects in mitochondrial fusion and neurodegenerative disease is particularly intriguing. In future studies, the pathophysiological mechanisms that underlie neurodegenerative diseases such as ADOA and CMT2A will hopefully be further dissected in appropriate animal models.

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DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
BAK | BAX | Dnm1 | DRP1 | FIS1 | FZO | Fzo1 | GDAP1 | Gem1 | MARCH5 | *MDM10* | *MDM12* | Mdm30 | Mdm31 | Mdm32 | Mdm33 | MFN1 | MFN2 | Mgm1 | *milton* | *Miro* | *MMM1* | NGF | OPA1 | SMAC | TIM23 | Ugo1
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
CMT2A | CMT4A

FURTHER INFORMATION
David C. Chan's homepage:
http://www.its.caltech.edu/~chanlab

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