Mitochondrial fission in apoptosis, neurodegeneration and aging

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A decline in mitochondrial function is well recognized in neurodegenerative diseases and aging, and is thought to play a causal role in their biology. Unfortunately, the molecular basis underlying this detrimental loss in mitochondrial function remains mysterious. Interestingly, mitochondria undergo frequent fission and fusion. This process is regulated by molecular machinery that has been highly conserved during evolution, including dynamin-related GTPases that manifest opposing effects. A balance between mitochondrial fission and fusion events is required for normal mitochondrial and cellular function. Emerging evidence indicates that mitochondria undergo rapid and extensive fission at an early stage during apoptosis. A clue that these new findings are of significance for the pathogenesis of neurodegenerative disease is provided by the observation that OPA-1, a dynamin-related GTPase regulating mitochondrial fusion, is mutated in humans with dominant optic atrophy, which is characterized by degeneration of retinal ganglion cells and childhood blindness. Loss of function of OPA-1, analogous to deficiency of its yeast homologue, Mgm1p, is expected to lead to mitochondrial fission, loss of mitochondrial DNA, respiratory deficits and an increase in reactive oxygen species. Here we review the molecular mediators controlling mitochondrial fission and fusion, and how death effector molecules may hijack this ancient machinery. A shift in the rate of mitochondrial fission or fusion may provide a new mechanistic explanation for the mitochondrial dysfunction in neurodegenerative diseases and normal aging, and may offer a new target for therapeutic intervention.

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Abbreviations

AIF	apoptosis-inducing factor
Drp1	dynamin-related protein 1
DOA	dominant optic atrophy
ER	endoplasmic reticulum
Fzo	fuzzy onion
GED	GTPase effector domain
Mfn	mitofusin
mtDNA	mitochondrial DNA
NTG	normal tension glaucoma
PARL	presenilin-associated rhomboid-like
PH	pleckstrin-homology
PTP	permeability transition pore
ROS	reactive oxygen species
SH3	Src homology 3

Introduction

The etiology of neurodegenerative diseases has long occupied the efforts of many research laboratories. But how neurons are lost during these devastating disorders, whose prevalence increases with age, remains poorly understood. Now new answers to this problem are emerging from recent studies of mitochondrial function. Mitochondria are best known as the powerhouse of the cell because of their important role in producing energy. Neurons rely heavily on mitochondrial function for their energy supply and specialized neuronal functions, including membrane ionic pumps, channel activity and synaptic transmission. In most cell types, including neurons, mitochondria are found as thread-like, tubular organelles that often branch and form connections [1]. This cable-like network allows respiratory complementation and mixing of mitochondrial DNA (mtDNA) [2,3].

Mitochondria undergo frequent fission and fusion that regulates their morphology, number and function [1]. Mitochondrial fission is required in dividing cells to ensure inheritance of mitochondria by daughter cells, but it is also important during differentiation, in response to new energy demands and as a result of toxin exposure [4]. Mitochondrial fission is also a normal process in post-mitotic cells such as neurons. When mitochondria divide, the tubular mitochondrial network splits into small, isolated organelles.

Mitochondrial fission events must be balanced with mitochondrial fusion events. Mitochondrial fusion serves to maintain a tubular mitochondrial network and optimal mitochondrial function. An interconnected mitochondrial network can facilitate transfer of the mitochondrial membrane potential from oxygen-rich to oxygen-poor cellular regions [5]. In *Drosophila*, mitochondrial fusion is a necessary event during development, where it regulates spermatogenesis. Specifically, blocking mitochondrial fusion by mutation of the fuzzy onion (Fzo) gene, results in male sterility [6]. Excessive mitochondrial fission and a lack of fusion results in breakdown of the mitochondrial network, loss of the mtDNA, respiratory defects and an increase in reactive oxygen species (ROS) [4].

Mitochondria contain an outer membrane and an inner membrane separated by an intermembrane space. The inner membrane is highly specialized and contains the electron transport chain that is responsible for oxidative phosphorylation and ATP synthesis. The inner membrane is folded into cristae that are bounded by narrow tubes known as crista junctions. These junctions may serve as diffusion barriers for soluble factors like cytochrome *c* or apoptosis-inducing factor (AIF) [7,8]. During mitochondrial fission, both membranes are constricted, and the mtDNA must be distributed into newly divided mitochondria. This process is accomplished by two distinct protein complexes involving large GTPases [4].

Here we discuss the molecular players involved in mitochondrial fission and fusion, and explore how altered regulation of this machinery may contribute to apoptosis, neurodegeneration and aging.

The mitochondrial fission machinery

The precise molecular mechanisms underlying mitochondrial division and fusion are not entirely understood. However, genetic studies in yeast, *Drosophila* and *Caenorhabditis elegans* have identified several key factors that are essential for this process. Remarkably, these factors are highly conserved throughout evolution, and homologues can be found in human and mouse.

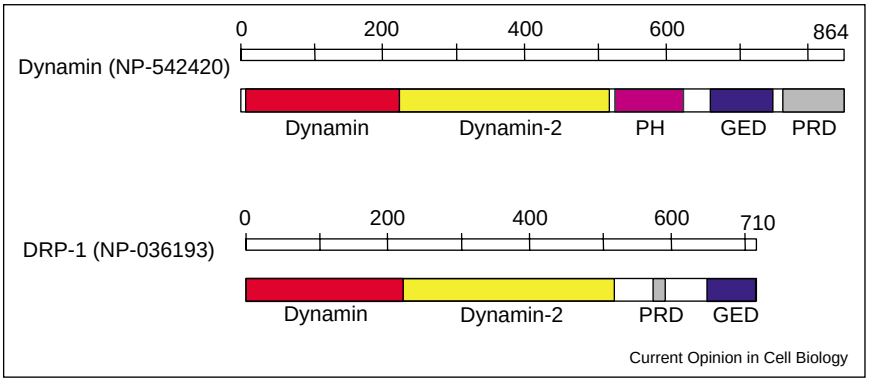
Mitochondrial fission in mammalian cells is regulated by dynamin-related protein 1 (Drp-1), a large GTPase [9,10]. Overexpression of Drp-1 triggers mitochondrial fission in *C. elegans* [11]. Conversely, knockdown of Drp-1 by RNAi silencing in *C. elegans* blocks mitochondrial outer-membrane scission, although inner-membrane scission still occurs [11]. Similarly, expression of a dominant-negative Drp-1 mutant, such as Drp-1K38A, that is unable to bind GTP results in a fused, interconnected mitochondrial network [10].

The amino acid sequence of Drp-1 shares significant similarity to that of dynamin (Figure 1) [12]. Dynamins are large GTPases that regulate vesicular traffic and endocytosis [13,14]. The domain structure of dynamin includes an N-terminal GTPase domain, a central Dynamin-2 domain, a pleckstrin homology (PH) domain for membrane targeting, a GTPase effector domain (GED) that regulates self-assembly and activation of GTP hydrolysis, and a C-terminal proline-rich region that serves as a binding site for proteins containing Src homology 3 (SH3) domains. Drp-1 shows significant homology to dynamin in the GTPase domain, central dynamin-2 domain and GED domain. Interestingly, however, the C-terminal PH domain of dynamin is missing in Drp-1. Instead, Drp-1 contains a unique proline-rich domain with an SH3 binding motif that is different from the proline-rich region in dynamin and thus may represent a unique regulatory domain.

The precise molecular functions of dynamin and Drp-1 are not yet known. However, two models have been proposed based on biochemical, genetic, and cell biological studies. The first model predicts that dynamin and Drp-1 act as mechanoenzymes, actively participating in membrane constriction and scission. This notion is supported by the observation that purified dynamin oligomerizes into rings and, upon addition of GTP, constricts lipid tubules *in vitro* [13]. Similar to dynamin, Drp-1 forms oligomeric complexes mediated by the C-terminal GED. In addition, purified Drp-1 oligomerizes into spirals *in vitro* [10].

The second model proposes that dynamin and Drp-1 do not act as force-generating GTPases, but rather as regulatory GTPases that recruit and activate an effector system that mediates membrane curvature and fission [15]. Possible effectors in this pathway include endophilins and related proteins. Endophilin 1, an SH3-domain-containing protein, binds to dynamin at its proline-rich

Figure 1



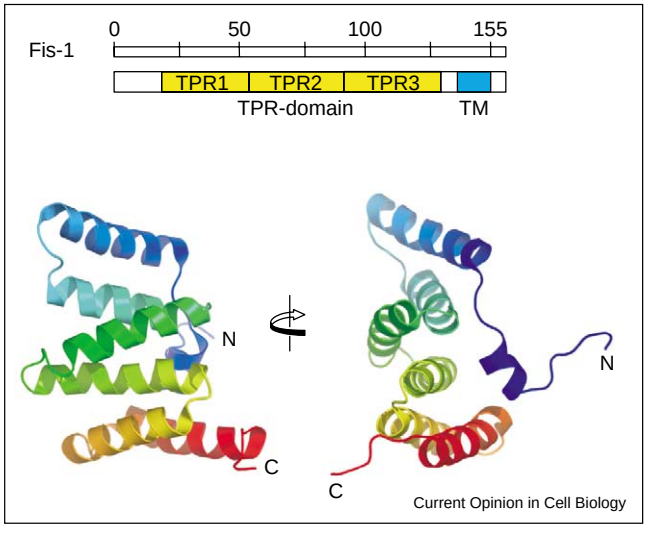
Domain comparison of dynamin and Drp-1. Abbreviations: dynamin denotes dynamin-like GTPase domain; dynamin 2 denotes dynamin-like central region; GED, GTPase effector domain; PH, pleckstrin homology domain; PRD, proline-rich domain. Notably the PRD domains in dynamin and Drp-1 are very different.

domain. Endophilin 1 is a presynaptic protein that cooperates with dynamin to mediate membrane curvature and vesicle budding during endocytosis [16,17]. Whether Drp-1 interacts in a similar fashion with endophilin-related proteins has not yet been explored.

Drp-1 normally resides in the cytoplasm but also accumulates at foci in the mitochondrial outer membrane that represent future fission sites [10]. Drp-1 lacks a mitochondrial targeting sequence and may be recruited to the mitochondrial outer membrane via Fis1, a small protein containing a transmembrane helix at the C terminus [18]. Fis1 may act as an adaptor in the assembly of high-molecular-weight fission complexes. The NMR structure of the soluble part of mFis1 has recently been solved and reveals a helical domain with three tetratricopeptide repeats that form a potential interaction site for WD-repeat-containing proteins (Figure 2). Although Drp-1 and Fis1 appear to be key regulators of mitochondrial fission, it is likely that other proteins that have yet to be identified participate in the effector system of mitochondrial fission. One candidate might be a mammalian analogue of the yeast protein Mdv1p, which contains WD repeats and regulates mitochondrial fission [19–22].

Very little is known about the signal-transduction pathways that initiate mitochondrial fission. However, several observations point toward Ca^{2+} as an important second messenger regulating mitochondrial fission. Firstly, dynamin activity appears to be regulated by the level of intracellular Ca^{2+} [13]. Secondly, Ca^{2+} release from the endoplasmic reticulum (ER) promotes the translocation of Drp-1 from the cytoplasm to the outer mitochondrial membrane [23••]. Thirdly, treatment with the Ca^{2+} ionophore, A23187, triggers mitochondrial fission in myoblasts and astrocytes [24]. Thus, it is possible that Ca^{2+} may directly activate Drp-1 and related fission effector molecules. Furthermore, the fission/fusion complexes might be regulated by phosphorylation; along these lines, the small GTPase Rab32, a protein kinase A anchoring protein, was recently found to trigger mitochondrial fission by an as-yet-unknown mechanism [25]. Figure 3 summarizes our model of mitochondrial fission events.

Figure 2



Domain scheme and 3D model of Fis1 based on NMR structure. The top shows the domain structure of Fis1. The bottom shows a ribbon representation of the mouse Fis1 (residues 15-132) structure (pdb_id:1iyg). Color-coded from N-terminus (blue) to C-terminus (red). The TPR domain of mouse Fis1 shares similarity with the peroxisomal targeting signal 1 receptor Pex5p/Pas10p (pdb:1hxi). Abbreviations: TM, transmembrane helix; TPR, tetratricopeptide repeat.

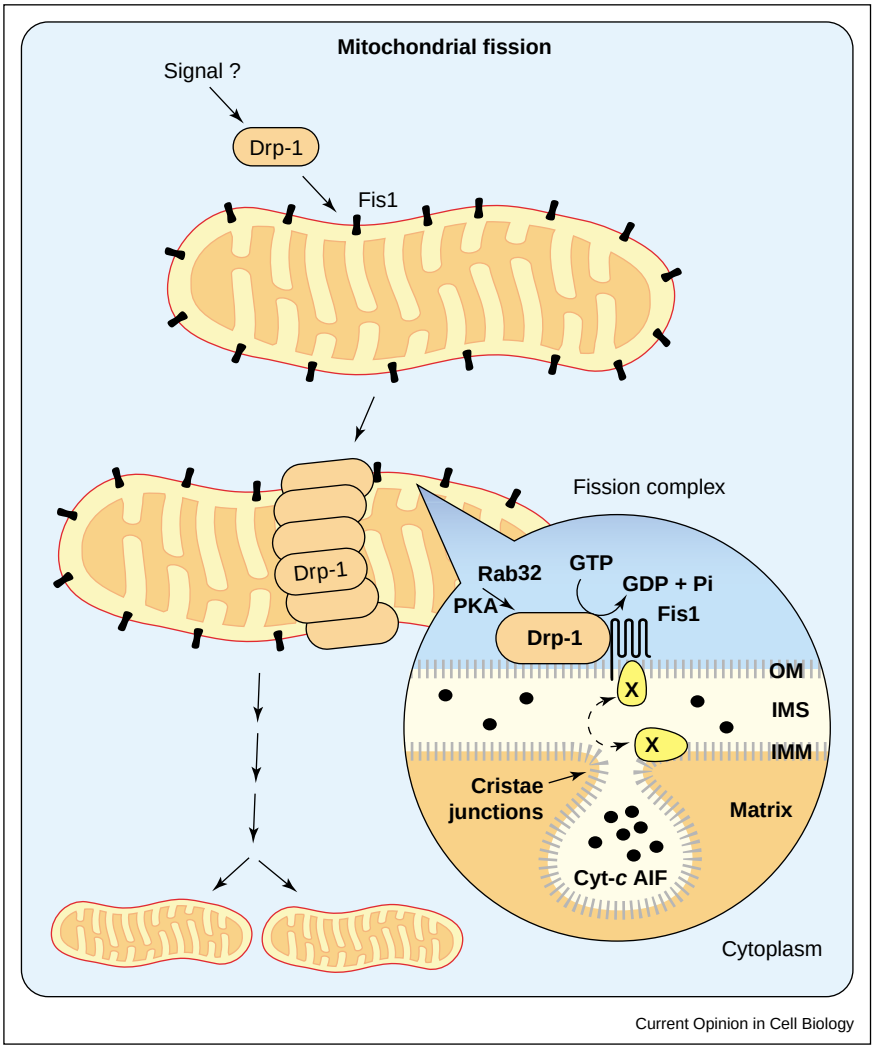
The mitochondrial fusion machinery

Although probably coordinated with the fission machinery, mitochondrial fusion appears to utilize a distinct set of evolutionarily conserved effector molecules. Among them are the large transmembrane GTPases, mitofusin (Mfn) 1 and 2 [26]. Mfn is comprised of an N-terminal dynamin-like GTPase domain and a C-terminal domain homologous to Fzo, which contains a transmembrane domain (Figure 4) [12,27]. In various cell lines, co-expression of Mfn2 with dominant-negative Drp-1 results in long, interconnected mitochondria [26].

OPA-1 is another factor implicated in mitochondrial fusion. OPA-1 is also a dynamin-related GTPase, but its function and subcellular localization are distinct from and in some senses opposite to Drp-1. The predicted domain structure of OPA-1 reveals an N-terminal mitochondrial targeting sequence, a transmembrane domain, a central dynamin-related GTPase domain, and a C-terminal helical domain (Figure 4) [12]. In yeast, deletion of the OPA-1 orthologue, MGM1, leads to extensive mitochondrial fragmentation, loss of mitochondrial DNA, deficits in respiration, and abnormal cristae structure [28]. It is possible that OPA-1 and Mfn cooperate in mitochondrial fusion as the yeast orthologues do [29], although this has not been formally demonstrated.

Most OPA-1 protein resides in the mitochondrial inner membrane, facing the intermembrane space (Figure 5) [30]. However, conflicting reports have suggested that OPA-1 is also present in the cristae, the intermembrane space, or even the mitochondrial outer membrane [31]. It is of note that different isoforms of OPA-1 with distinct molecular weights have been described. Thus, it is possible that disparate OPA-1 isoforms have distinct subcellular localizations and functions [31]. Along these lines, new findings have emerged from studies in yeast suggesting that OPA-1 might be activated by proteolytic removal of its transmembrane domain by rhomboid serine proteases [32•,33•] (Figures 4 and 5). Rhomboid proteases in *Drosophila* are implicated in EGF receptor signaling, inducing proteolytic cleavage and liberation of the

Figure 3



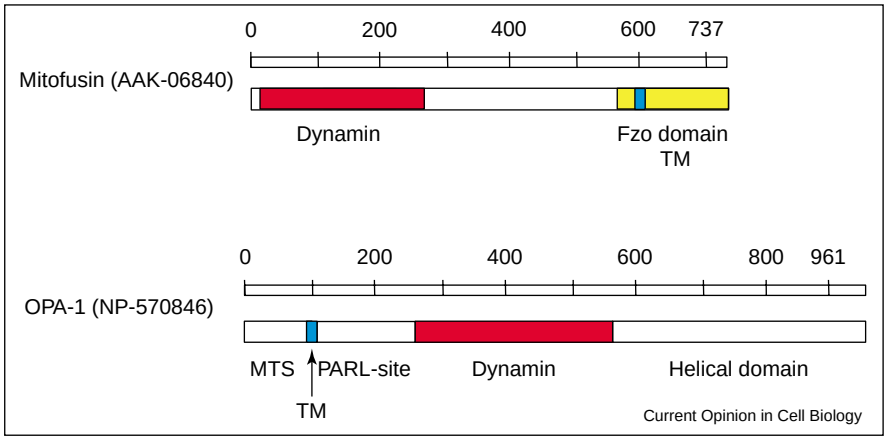
Schematic model of mitochondrial fission. Drp-1 resides in the cytoplasm but, upon activation by an unknown signal, translocates and binds to the mitochondrial outer membrane via Fis1, where it assembles into a large oligomeric ring-like complex. Drp-1 may either act as mechano-enzyme involved in membrane constriction and fission or as a regulatory GTPase involved in the activation of as-yet-unidentified effector molecule(s) (labeled X in the figure). Activated Drp-1 complexes may convey signals to (or from) the inner membrane to coordinate outer and inner mitochondrial membrane fission. Rab32, a PKA-anchoring protein, might be a regulator of the mitochondrial fission machinery. Abbreviations: IMM, inner membrane; IMS, intermembrane space; OM, outer membrane.

EGF receptor ligand precursor that is normally anchored in an inactive form in cellular membranes [34]. In yeast, the rhomboid protease Rbd1p/Pcp1p, an integral protein of the mitochondrial inner membrane, cleaves Mgm1p at its transmembrane domain, thus producing a shorter isoform [32•,33•]. Expression of catalytically inactive *rbd1* or *mgm1* or of a cleavage-resistant *mgm1* mutant results in mitochondrial fragmentation [32•,33•]. Thus, membrane anchoring of Mgm1p might interfere with its dynamin-like membrane-remodeling activity. The short, soluble form of Mgm1p may be part of the active form and might mediate formation of cristae and their junctions (Figure 5). Rhomboid proteases are conserved throughout evolution and the human homologue of Rbd1p is the presenilin-associated rhomboid-like (PARL) protease. Intriguingly, the Rbd1p recognition sequence is conserved and also present in OPA-1. It remains to be shown, however, whether PARL cleaves and activates human OPA-1.

Mitochondrial fission in apoptosis

Mitochondria are central players in the initiation and execution of apoptosis and other forms of cell death [35]. Mitochondrial dysfunction in cell death is characterized by a decline in mitochondrial membrane potential ($\Delta\psi_m$), respiratory defects, an increase in ROS production,

Figure 4



Predicted domain structure of Mitofusin and OPA-1. Abbreviations: Dynamin, Dynamin-like GTPase; Fzo, Fuzzy Onion domain; MTS, mitochondrial targeting sequence; PARL, Presenilin-associated rhomboid-like protease recognition site; TM, trans-membrane helix.

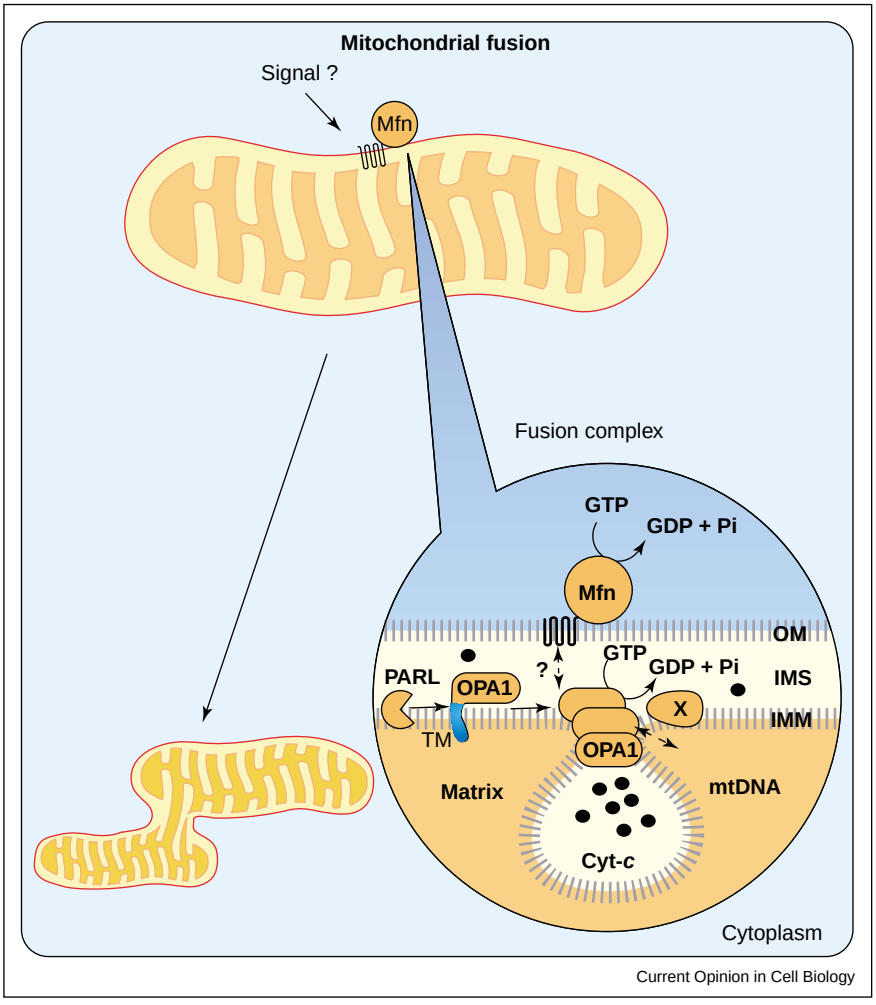
changes in ATP levels and release of apoptogenic factors, including cytochrome *c* and AIF.

Although the release of apoptogenic factors from mitochondria constitutes a key event in cell death, the exact molecular basis of this fundamental process remains unknown. Pro- and anti-apoptotic members of the Bcl-2 family of proteins are important regulators of mitochondrial function [35]. They form either homo- or heterodimers, and the ratio of pro- versus anti-apoptotic complexes determines whether cells live or die. BH3-domain-only Bcl-2 family members, including Bid, Bad and Bim, as well as multidomain members Bax and Bak, have been shown to increase outer mitochondrial membrane permeability [36]. However, the molecular mechanism of this increase in permeability is still unknown. One view proposes that Bax and related proteins form pores. Consistent with this notion, the crystal structure of Bax displays similar features to the pore-forming domain of diphtheria toxin. In addition, Bax has been shown to exhibit channel activity in artificial lipid bilayers. Another view suggests that Bax may interact at the mitochondrial outer membrane with the voltage-dependent anion channel that is part of the permeability transition pore (PTP). Opening of the PTP has been linked to apoptosis and the release of apoptogenic factors [35]. In the same context, Scorrano *et al.* [37••] recently proposed a two-step model for cytochrome *c* release. This model involves, first, a profound remodeling of the mitochondrial inner membrane to mobilize 85% of the cytochrome *c* that is trapped in the cristae and, second, a tBid- and Bax-mediated translocation of cytochrome *c* across the mitochondrial outer membrane. Moreover, tBid is associated with opening of the cristae junctions and an increase in inner-membrane connectivity. tBid has also been shown to exhibit lipid-translocase activity as well as the ability to alter membrane curvature, at least *in vitro* [38]. Thus, it is possible that tBid directly alters membrane curvature *in vivo* or regulates targets that are involved in cristae remodeling. It is therefore interesting to speculate that tBid may regulate the activity of fission and fusion effector molecules.

Emerging evidence suggests that mitochondrial fission might be an important part of the central cell death machinery. Several reports on cell lines indicate that mitochondria undergo rapid and excessive fission evoked by diverse stimuli early in the apoptotic process (Figure 6) [39–42]. Furthermore, apoptotic mitochondrial fission appears to be independent of effector caspases.

Although there is mounting evidence that mitochondria undergo fission in apoptosis, the molecular pathways mediating this process are poorly understood. Interestingly, Youle and colleagues recently found that Drp-1 participates in apoptotic mitochondrial fission [43]. Specifically, Drp-1 translocates from the cytosol to defined foci on the mitochondrial outer membrane at the onset of apoptosis. Importantly, dominant-negative Drp-1 blocks staurosporine-induced mitochondrial fission, loss of $\Delta\psi_m$, cytochrome *c* release and cell death [43]. Conversely, Martinou and colleagues have shown that forced expression of human Fis1 triggers mitochondrial fission, cytochrome *c* release and subsequent apoptosis [44•]. Additionally, Bcl-xL, a potent cell-death inhibitor, blocks cytochrome *c* release and apoptosis, but does not interfere with Fis1-induced mitochondrial fission, thus demonstrating that mitochondrial fission and cell death can be separable events [44•]. Furthermore, Karbowski *et al.* recently reported that Bax, a pro-apoptotic member of the Bcl-2 family, colocalizes with Drp-1 and Mfn2 at mitochondrial fission sites early in the apoptotic process

Figure 5



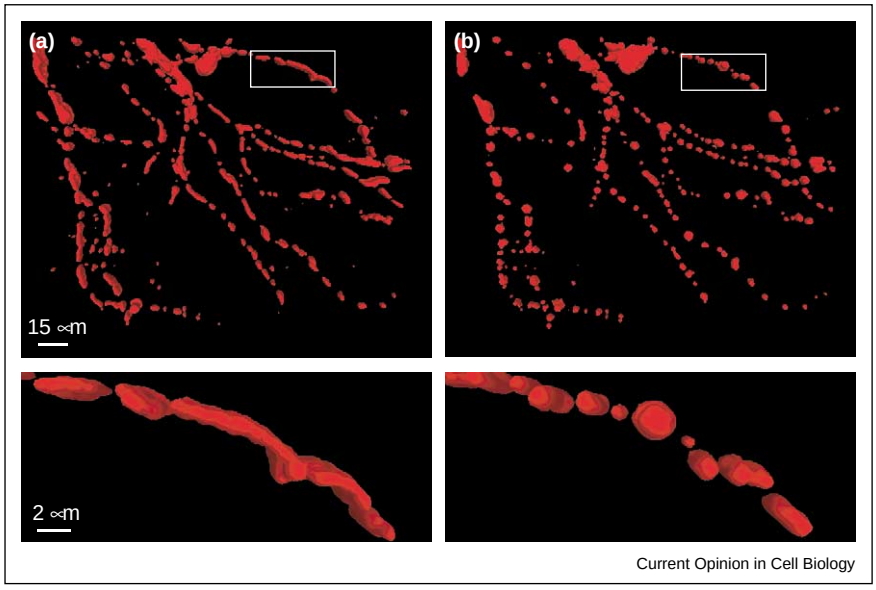
Schematic model of mitochondrial fusion. The outer membrane protein, Mfn, a large GTPase, and OPA-1, a dynamin-related GTPase, regulate mitochondrial fusion in mammalian cells. It is possible that Mfn and OPA-1 interact. OPA-1 is primarily located in the mitochondrial inner membrane and becomes activated by proteolytic cleavage of its transmembrane domain, which is mediated by PARL. Once released from the membrane, OPA-1 may oligomerize and assemble into large complexes that mediate cristae remodeling, formation of cristae junctions and maintenance of mtDNA. OPA-1 is likely to interact with and regulate yet unknown factors (labeled X in the diagram). Abbreviations: Cyt-*c*, cytochrome *c*; IMM, inner membrane; IMS, intermembrane space; OM, outer membrane; TM, transmembrane region.

[45••]. In addition, a dominant-negative Drp-1 mutant attenuates Bax-induced mitochondrial fission [43], but does not affect Bax clustering into foci on the mitochondrial outer membrane [45••]. Interestingly, the regions where Bax accumulates in the presence of dominant-negative Drp-1 exhibit signs of incomplete mitochondrial fission [45••]. Thus, these findings link pro-apoptotic members of the Bcl-2 family to the mitochondrial fission and fusion machinery and suggest that this machinery plays a key role in the cell-death program.

As mentioned above, Ca^{2+} may be important in mitochondrial fission. Additionally, an increase in free Ca^{2+} has been implicated in mitochondrial dysfunction during cell death [46]. New findings by Shore and colleagues establish a novel form of cross-talk between ER-derived Ca^{2+} signals, Drp-1, and mitochondrial fission. BAP31, an ER membrane protein, is cleaved by caspase-8 during apoptosis, yielding a 20-kDa fragment (p20) that induces Ca^{2+} release from the ER, which in turn promotes Drp-1-dependent mitochondrial fission [23••]. Importantly, preventing Ca^{2+} efflux from the ER blocks p20-induced mitochondrial fission [23••]. These results suggest that intracellular Ca^{2+} signaling might be an important regulator of mitochondrial membrane dynamics.

Clearly, mitochondrial fission *per se* does not result in cell death. However, cell death does not occur without mitochondrial fragmentation, at least in the paradigms so far investigated. Thus one is left with the question: what is

Figure 6



Mitochondrial fragmentation within neuronal processes. Neuritic processes of a cortical neuron are illustrated. They have been labeled by transfection with the DsRed2–Mito expression vector that encodes a fusion peptide consisting of *Discosoma* sp. red fluorescent protein and the mitochondrial targeting sequence of human cytochrome *c* oxidase subunit VIII, thereby labeling the mitochondrial inner membrane. The neuron was treated with 10 μM nocodazole, and changes in mitochondrial morphology were monitored by time-lapse fluorescence deconvolution microscopy with 3D image reconstruction (software: Slidebook 4.0, Intelligent Imaging Innovations; Volocity 2.0, Improvision). **(a)** Mitochondrial morphology five minutes after nocodazole exposure. Mitochondria exhibit tubular and interconnected morphology, similar to their baseline appearance. **(b)** Mitochondrial fission 35 minutes after nocodazole exposure. Mitochondria fragment into punctiform organelles of variable size.

the exact role of mitochondrial fission in the central cell-death pathway? Mitochondrial fission may represent an ancient stress response aimed at repairing cell injuries. Mitochondria are thought to originate from primitive α-proteobacteria, and the GTPase FtsZ mediates division in these bacteria [47]. Intriguingly, FtsZ forms ring-like structures around fission sites. Recently, mitochondrial fission in apoptosis has been proposed to resemble bacterial sporulation [48]. We further suggest that apoptotic effectors, like Bax or tBid, modulate the mitochondrial fission machinery, which results in accelerated fission and opening of the inner membrane cristae junctions. This event may facilitate the loss of cytochrome *c* from mitochondria and the progression of cell death (Figure 7).

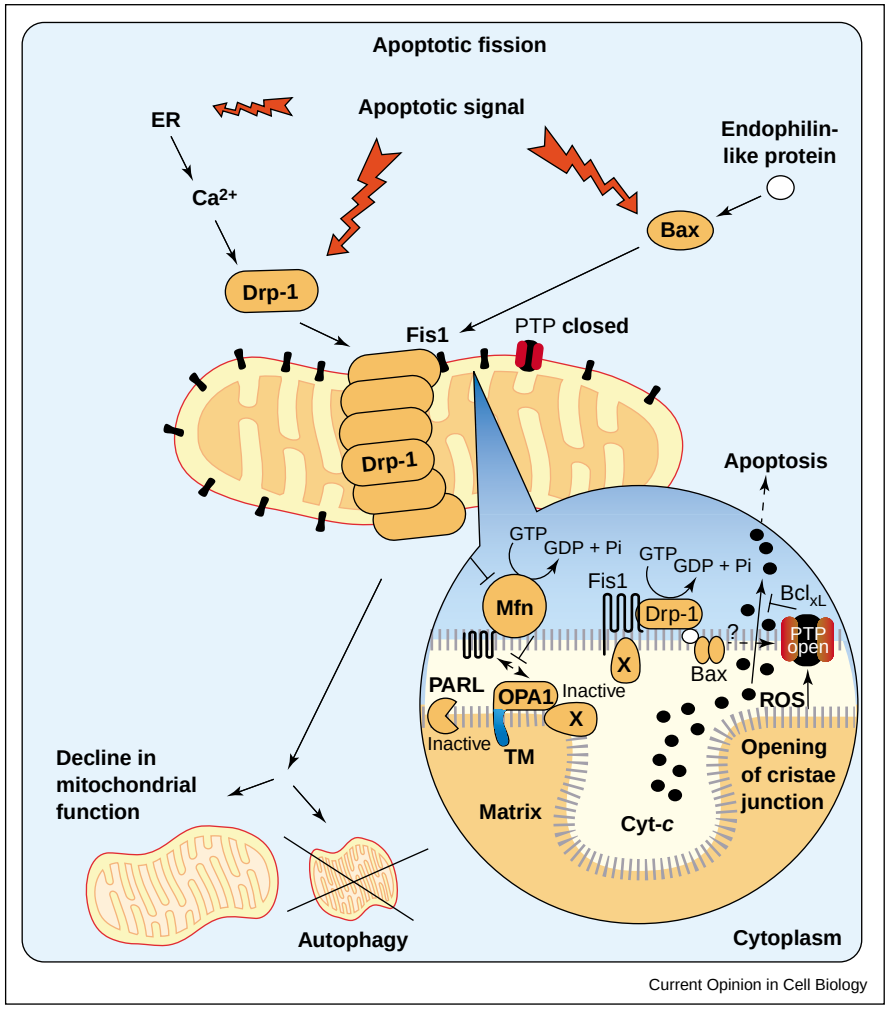
Mitochondrial fission in neurodegenerative disease

The precise mechanisms underlying neuronal cell loss in most neurodegenerative disorders remain elusive. However, hints from genetic and clinical data in humans suggest that excessive mitochondrial fission may mediate some forms of neurodegenerative disease. Intriguingly, new findings indicate that mutations in OPA-1 cause dominant optic atrophy (DOA), the most common form of autosomal inherited optic neuropathy, which leads to retinal ganglion cell death and progressive loss of vision in children [49,50]. Hot spots of OPA-1 mutations cluster in the GTPase domain and are likely to abolish GTP binding. Additionally, monocytes from DOA patients manifest abnormal mitochondrial networks [50].

Downregulation of OPA-1 by small interfering (si)RNA in HeLa cells results in mitochondrial fragmentation, loss of $\Delta\psi_m$ and cytochrome *c* release [51•]. It has not been tested whether mtDNA is lost in this system. Interestingly, a decrease in OPA-1 in these cells leads to striking remodeling of the mitochondrial cristae with formation of unusual vesicle-like structures [51•]. As mentioned above, OPA-1 may control the structure and dynamics of cristae. Opening of the cristae junctions occurring as a result of loss of OPA-1 activity, therefore, could possibly be a prerequisite for cytochrome *c* release.

It is expected that OPA-1 mutations would lead to destabilization of the mitochondrial inner membrane, with consequent respiratory defects, an increase in ROS and reduced energy levels in retinal ganglion cells, which are severely affected in DOA. Prolonged or excessive breakdown of the mitochondrial network may lead to unequal energy distribution. It is of note that normal tension glaucoma (NTG) clinically resembles DOA. However, the onset of retinal ganglion cell degeneration and vision loss in NTG patients is more sporadic and occurs later in life and often only in one eye, unlike DOA [52]. It

Figure 7



Schematic model of apoptotic mitochondrial fission. Ca^{2+} signals from the ER or other apoptotic stimuli trigger the translocation of Drp-1 and Bax to the mitochondrial outer membrane. Bax interacts with endophilin-like molecules that contain an SH3 domain, such as Bif-1. Bax, endophilins and Drp-1 are postulated to form a large complex at sites of mitochondrial fission. Drp-1 may convey a conformational change to Bax and associated molecules, thereby facilitating an increase in outer membrane permeability and translocation of cytochrome *c* across the cytosol. Inactivation of PARL or OPA-1, a factor involved in mitochondrial fusion, may lead to the opening of cristae junctions and the liberation of apoptogenic factors like cytochrome *c* or AIF. This process may lead to disintegration of mitochondria and their removal by autophagy. Abbreviations: cyt-*c*, cytochrome *c*; TM, transmembrane region.

remains possible that OPA-1 becomes inactivated in NTG by a mechanism other than mutation.

OPA-1 mutation in humans leads to progressive loss of vision early in life [49,50,52]. Why are retinal ganglion cells primarily affected and not other cell types? One possible reason for the selective vulnerability is that retinal ganglion cells are particularly sensitive to ROS. Another possibility is that retinal ganglion cells have fewer buffer systems to compensate for loss of OPA-1 function. Of note, the greatest number of mitochondria in retinal ganglion cells is found in the nonmyelinated axon hillock area, where action potentials are generated. Thus, we postulate that mitochondrial defects may have severe consequences for electrophysiological activity in retinal ganglion cells. Moreover, epigenetic factors, such as daily exposure to ultraviolet rays, may also contribute to the susceptibility of retinal ganglion cells to loss of OPA-1 function.

The biochemical function of OPA-1 is unknown, but there are several possibilities. Firstly, OPA-1 might act in the formation and stabilization of mitochondrial cristae as well as in the maintenance of mtDNA. Opening of the narrow cristae junctions, and hence mobilization of sequestered cytochrome *c*, might require inactivation of OPA-1, which may be part of the apoptotic program. To effect this change, OPA-1 may interact with apoptotic

effectors like tBid [37••]. A second possibility is that OPA-1 acts as a mitochondrial fusion factor, perhaps by interacting with outer membrane fusion factors such as Mfn. OPA-1 might also modify the inner mitochondrial membrane curvature, thereby generating a fusion-competent configuration. Lastly, OPA-1 might act as an inhibitor of mitochondrial fission. As mentioned above, disparate OPA-1 isoforms have been identified. Thus, it is possible that OPA-1 is bi-or multi-functional and that its activity depends on which isoform predominates.

As alluded to earlier, it is possible that OPA-1 is cleaved and activated by PARL, which is found in the mitochondrial inner membrane [32•,33•,53]. Mutations in the presenilin-1 or -2 gene predispose to some forms of inherited early-onset Alzheimer's disease [54]. It will be interesting to test whether PARL cleaves and activates human OPA-1, and whether this process is inhibited by mutant presenilins, thereby promoting mitochondrial fragmentation. Alzheimer's disease is characterized by progressive impairment of cognitive skills and memory loss due to neuronal cell demise, especially in the hippocampus and cortex, where amyloid plaques accumulate. Degenerating neurons exhibit neuritic Tau hyperphosphorylation, increased Ca^{2+} levels, excessive free radical formation and mitochondrial dysfunction [54]. The basis of mitochondrial dysfunction in Alzheimer's disease is unknown. It will be of interest to test whether an increased rate of mitochondrial fission might be part of the underlying mechanism of neurodegeneration in this devastating disorder.

Parkinson's disease is marked by the progressive loss of dopaminergic neurons in the substantia nigra. Degenerating neurons manifest an increase in ROS production and mitochondrial dysfunction [54]. Environmental toxins and the pesticide rotenone are thought to increase the risk for Parkinson's disease [55]. In animal models, the toxin 1-methyl 4-phenyl-1,2,3,6-tetra hydropyridine (MPTP) evokes a Parkinson's-like disease and impairs respiratory complex I, resulting in ATP depletion and oxidative stress [56]. It is of note that classical mitochondrial toxins such as FCCP trigger mitochondrial fission, but it is currently unknown whether the mitochondrial toxins associated with the Parkinson's disease phenotype trigger fission as well.

Thus, in future experiments it will be important to test whether increased rates of mitochondrial fission might at least in part be involved in the pathogenesis of these as well as other neurodegenerative disorders.

Mitochondrial fission and fusion in aging

Aging is associated with a marked decline in mitochondrial function, characterized by a decrease in oxidative phosphorylation and ATP synthesis, an increase in mtDNA mutations, an increase in abnormal mitochondrial cristae structures and a marked rise in free radical production, all of which may predispose to neurodegenerative disorders [57]. In aged heart and other tissues, mitochondria are often found in fewer numbers. Thus, one can speculate that deficits in mitochondrial fission and fusion rates might account for a progressive, age-associated deterioration of mitochondrial function. Mitochondrial fusion complements mtDNA mutations [2] and thus could be seen as a defense against aging. Also, fused mitochondrial filaments can compensate for uneven oxygen supply and can distribute energy more evenly throughout the cell [3,5]. An increase in oxidative stress, as occurs during aging, may in itself evoke breakdown of the mitochondrial network, thus setting off a self-reinforcing cycle. This may lead to loss or mutation of mtDNA that remains uncomplemented, leading to further oxidative stress. Clearly, future experiments are needed to test this hypothesis.

Conclusions

Here we have discussed the possibility that mitochondrial fission might be an underlying mechanism in several neurodegenerative disorders. Moreover, defects in the mitochondrial fission/fusion machinery may contribute to the decline in mitochondrial function during aging. However, several fundamental questions remain to be answered. What are the missing factors that interact with key mitochondrial fission/fusion effectors such as Drp-1 and OPA-1? What is the structure and function of Drp-1 and OPA-1? How are fission and fusion events of the inner and outer mitochondrial membrane coordinated? What are the signal transduction pathways that trigger mitochondrial fission and fusion processes? What is the precise mechanism and role of mitochondrial fission in apoptosis and neurodegenerative diseases? Clearly, exciting years of research lie ahead to address these questions. Components of the mitochondrial fission machinery may represent new and important targets for the potential treatment of neurodegenerative disorders and the extension of lifespan by delaying the aging process.

Update

Since this review was submitted for publication, four papers relevant to the review topic have been published. First, a review by Nunnari and colleagues highlights the machinery of mitochondrial fission and fusion, with a particular focus on known yeast components, and connects these processes to apoptosis and mtDNA inheritance [58]. Another review, by Karbowski and Youle, dissects the involvement of mitochondrial fission and fusion proteins in cell death and survival, while proposing yet unexplored possibilities for further links between these phenomena [59]. Still another review, by Scorrano, details the role of intracellular Ca^{2+} as a potential integrator of mitochondrial fission and cell death, emphasizing the hypothesis that Ca^{2+}-activated mitochondrial fission is not sufficient for, but sensitizes the cell to,

apoptosis [60]. Finally, Yoon *et al.* [61] demonstrate that human Fis1 interacts with Drp-1 and, like Martinou and colleagues [44•], suggest that Fis1 may be the limiting component of the mitochondrial fission pathway, as enforced expression of this protein alone promotes the fission event in mammalian cells [61].

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