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REVIEW

Splitting up the powerhouse: structural insights into the mechanism of mitochondrial fission

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Abstract Mitochondria are dynamic organelles whose shape is regulated by the opposing processes of fission and fusion, operating in conjunction with organelle distribution along the cytoskeleton. The importance of fission and fusion homeostasis has been highlighted by a number of disease states linked to mutations in proteins involved in regulating mitochondrial morphology, in addition to changes in mitochondrial dynamics in Alzheimer's, Huntington's and Parkinson's diseases. While a number of mitochondrial morphology proteins have been identified, how they co-ordinate to assemble the fission apparatus is not clear. In addition, while the master mediator of mitochondrial fission, dynamin-related protein 1, is conserved throughout evolution, the adaptor proteins involved in its mitochondrial recruitment are not. This review focuses on our current understanding of mitochondrial fission and the proteins that regulate this process in cell homeostasis, with a particular focus on the recent mechanistic insights based on protein structures.

Keywords Mitochondria · Fission · Dynamin-related protein 1 · Adaptors · Lipids · Outer mitochondrial membrane · Endoplasmic reticulum

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Introduction

Mitochondria are integral to cellular function and are responsible for energy production in eukaryotes, synthesis of metabolites, phospholipids and heme, and maintenance of calcium homeostasis [1–3]. While these are well-established functions, mitochondria have recently been shown to be anti-viral signalling platforms and contribute to apoptotic activation and cell death [4–6]. Given the diverse role of mitochondria in cellular physiology, it is not surprising that alterations in mitochondrial function are associated with disease states including Alzheimer's, Huntington's Parkinson's diseases [7–12]. These and other diseases (e.g. Charcot–Marie Tooth 2A disease and optic atrophy) can be attributed to defects in known morphology proteins [13]. The opposing processes of fission and fusion maintain mitochondrial morphology and it is this equilibrium that ensures maintenance of mtDNA and metabolic mixing, bioenergetic functionality and organelle number [14, 15].

An imbalance in fission and fusion events can lead to a marked shift in morphology and viability of the organelle. Together these processes maintain the steady-state mitochondrial morphology needed for normal cell function, yet individually fission and fusion perform distinct roles. Mitochondrial fusion maintains a homogenous organellar population and ensures complete complementation of mtDNA [14]. In mammals, fusion is controlled by large GTPases on both the inner (OPA1) and the outer (MFN1/MFN2) mitochondrial membranes [16, 17]. The signals that trigger activation and co-ordination of a double membrane fusion event are currently unknown. As mitochondria cannot be made de novo, mitochondrial fission facilitates inheritance of mitochondria in dividing cells; but left unregulated, fission can lead to a heterogeneous

population of organelles with disproportional mtDNA distribution, increased capacity to generate reactive oxygen species and altered viability of the organelle [18, 19]. Mammalian cells that are unable to undergo fission harbour mtDNA in irregularly clustered nucleoids, contributing to a non-uniform cristae structure that encloses the pro-apoptotic protein cytochrome *c*—resulting in a delay in programmed cell death [20]. Fission is one of the many important steps in mitophagy (a mitochondrial-specific pathway of organelle turnover) since it is stimulated by activation of pro-fission proteins [21, 22].

Dynamin-related protein 1 (Drp1)

Dynamins are a large superfamily of GTPases that have a number of fundamental roles in cellular homeostasis, including membrane fission events, anti-viral signalling, chloroplast biogenesis and plant cell plate formation [23, 24]. The master fission mediator conserved throughout evolution is dynamin-related protein (DRP) family member, Drp1 (or Dnm1 in yeast) [25, 26]. Drp1-mediated fission in different organisms is supported by the specialised architecture of adaptor proteins, including Fis1, Mff, MiD49 and MiD51 in mammals and Mdv1/Caf4 in yeast, as well as cytoskeletal and endoplasmic reticulum (ER) contacts [27, 28]. The importance of Drp1 and fission in mammalian physiology has been recently highlighted. A patient harbouring a heterozygote dominant-negative Drp1 allele presented with broad metabolic defects (both mitochondrial and peroxisomal), abnormal brain development, optic atrophy and died 37 days after birth [29]. In addition, Drp1 knockout in mice results in hypoplasia of the forebrain and is ultimately embryonic lethal [30, 31]. However, mouse embryonic fibroblasts isolated from Drp1 knockout mice are viable in culture, emphasising the hierarchical effect of loss of fission in cell lines, tissue or whole organism. In the case of the Drp1 knockout mouse, global loss leads to specific nervous system impairment. This is augmented in highly polarized cells such as neurons as they have an absolute reliance on oxidative phosphorylation with limited capacity for glycolysis [32, 33].

Post-translational regulation of Drp1

Drp1 can be modified by variety of proteins and cellular signals, and each of these can affect activity in different ways. These modifications include phosphorylation, S-nitrosylation, ubiquitination and sumoylation [34–36]. Cdk1/cyclin phosphorylates Drp1 at S585 and is thought to be an important step during mitosis as it can lead to increased fragmentation to ensure efficient segregation of mitochondria into daughter cells [37]. Phosphorylation of Drp1 at S367 by cAMP-dependant protein kinase A (PKA) inhibits the protein's GTPase activity and impairs fission [38, 39]. Dephosphorylation of Drp1 is equally important in regulation of the protein—calcineurin removes the phosphate at S367 and in turn causes an increase in fragmentation following an increase in intracellular calcium levels [39]. Recently mitochondrial fission was shown to be required for RAS-induced cellular transformation. In this case, Erk2 phosphorylates Drp1 at S616 to activate fission. Furthermore, expression of a Drp1 S616A mutant that prevents phosphorylation, and blocks Ras-induced tumour growth [40, 41]. The mechanism by which mitochondrial fission is required for cell transformation and tumorigenesis is not clear but may involve aspects of mitochondrial reprogramming.

Drp1 domain structure

The domain structure of Drp1 consists of an amino terminal GTPase domain, a middle domain and the GTPase effector domain (GED), decorated with further structural elements that direct dynamin function (Fig. 1) [42–44]. Drp1 is predominantly cytosolic; however, a considerable quantity is found at mitochondria, specifically in foci that represent future or previous sites of fission [45]. Upon recruitment to mitochondrial constrictions, Drp1 polymerises into spirals and through GTP-dependent conformational changes, constricts the organelle leading to membrane scission [46]. Drp1 is also involved in peroxisomal fission, recruited to the similarly dynamic organelle by adaptor proteins Mff and Fis1 [47, 48].

Drp1 and Dnm1 share the same structural features and consist of a GTPase domain, a bundle-signalling element (BSE), a stalk, and an Insert B (InsB) domain (Fig. 1). Unlike dynamins, Drp1 and Dnm1 lack a lipid-interacting pleckstrin homology (PH) domain or a compensating transmembrane region such as those found in the other morphology-mediating GTPases OPA1 and MFN1/2 [49]. Instead they harbour a variable InsB region that was predicted to interact with mitochondrial adapter proteins [50, 51]. Additionally, while the solved structure of the PH domain reveals a compact, β-strand-rich fold, loosely attached to the dynamin stalk, InsB is predicted to be largely unstructured [42, 43, 50] (Fig. 1). Recently, it was demonstrated that InsB in Drp1 is not absolutely required for fission activity but can modulate Drp1 oligomerisation and activity through post-translational modifications [50].

Cryo-electron microscopy of Dnm1-decorated liposomes has shed light on its oligomerisation and constriction mechanisms [52]. Both proteins assemble on liposomes in the absence of nucleotide in a helical fashion; however, while dynamin forms a one-start helix, the more loosely packed Dnm1 forms a two-start helix, resulting in a larger

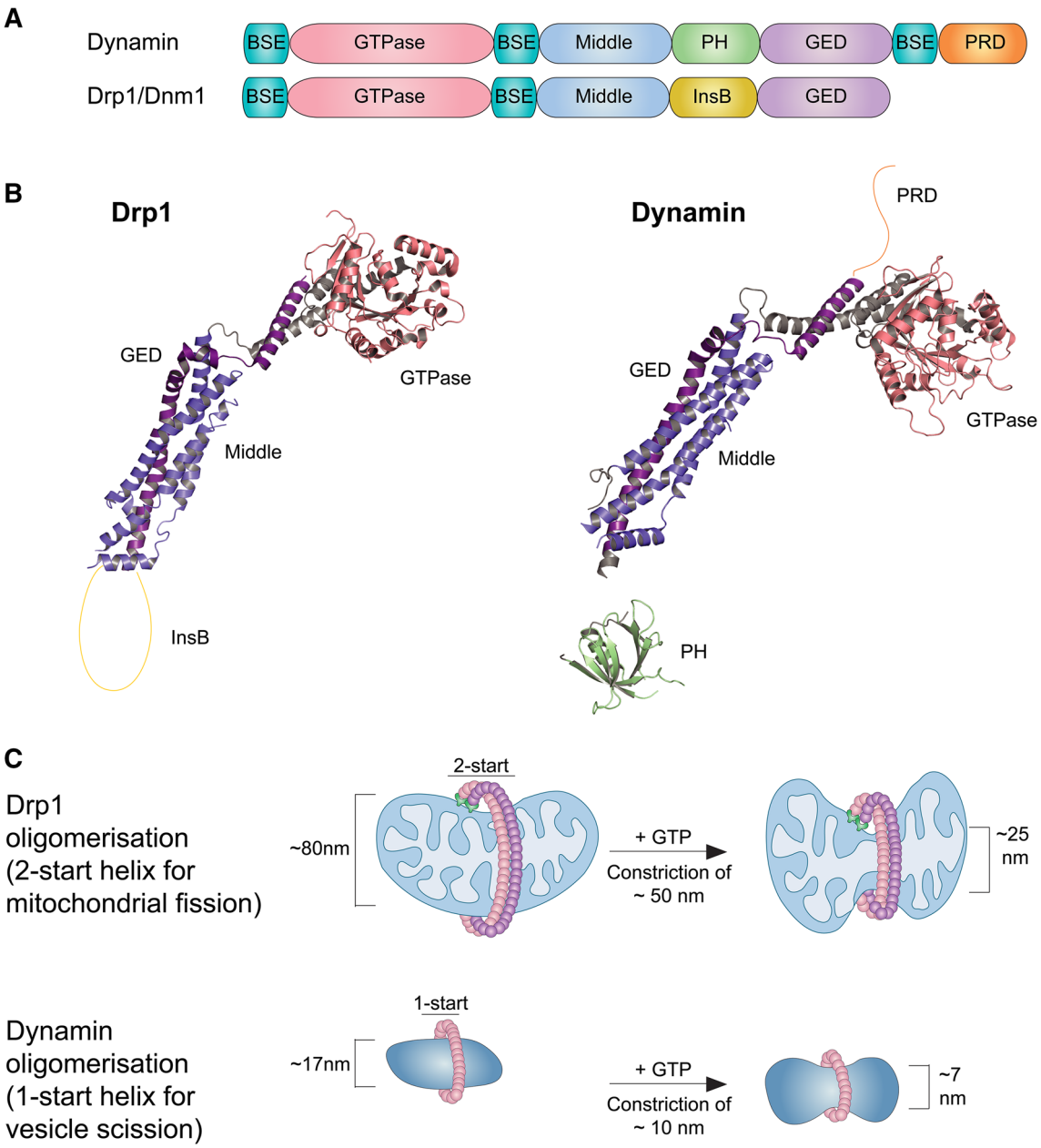

Fig. 1 Drp1/Dnm1 and dynamin assembly and contractile mechanisms. **a** Structural motifs present in Drp1/Dnm1 and dynamin. *BSE* bundle signalling element, *GTPase* GTPase domain, *Middle* middle domain, *PH* pleckstrin homology domain, *InsB* insert B, *GED* GTPase effector domain, *PRD* proline rich domain. **b** Crystal structures of Drp1 and dynamin (PDB accession numbers: Drp1: 4BEJ; dynamin: 3ZVR). **c** Models of 2- and 1-start helices formed by Drp1 and dynamin, respectively. While dynamin interacts with the lipid bilayer of membranes directly (via a PH domain), Drp1 requires the presence of membrane adapter proteins at the mitochondrial surface

helical pitch as well as a larger structure diameter (Fig. 1) (129 nm for Dnm1, 50 nm for dynamin) [52]. Additionally, while the three-dimensional reconstruction of the dynamin oligomer identified an interaction with the underlying lipid bilayer, a 3–4 nm gap was observed between Dnm1 and the lipid tubule, indicating that the InsB region that substitutes in the position of the PH domain of dynamin-1 does not directly interact with the membrane [52]. While the typical diameter of the neck of a clathrin-coated vesicle of 40 nm can be encircled by around 14 dynamin dimers, the larger mitochondrial constriction sites of around 110 nm require a more substantial constriction and is calculated to require 48 Dnm1 dimers or 24 Drp1 tetramers per turn of the helix [44, 52–54]. Analysis of the conformational changes produced by GTP hydrolysis revealed notably larger decrease in the helical diameter of Dnm1 than what has been

described for dynamin [55, 56]. Dnm1 produces a 50-nm outer membrane diameter decrease, as compared to 10 nm observed for dynamin, a constriction associated with increased axial spacing resulting from a sliding mechanism between adjacent strands. The proximity between two single opposing lipid surfaces required for spontaneous membrane fission has been calculated to be 1–2 nm [57, 58]. As the average luminal diameter of Dnm1-lipid tubules falls outside this range, it is arguable whether GTP hydrolysis of Dnm1 alone is sufficient to cause membrane scission, giving rise to the possibility that membrane receptors for Dnm1/Drp1 have a function in promoting further oligomer constriction in addition to their role in recruitment [52].

The crystal structure of Drp1 was recently solved and reveals important insight into oligomerisation interfaces and mechanisms [44]. Similar to what has been previously shown for dynamin, the stalk domains of Drp1 mediate dimerisation and higher-order oligomerisation via three interfaces [42–44]. The crystal structure identified a novel fourth assembly interface on the stalk of Drp1, resulting in a parallel stacking of the dimer in the crystal lattice. This Drp1-specific interface was proposed to assemble two neighbouring Drp1 filaments, stabilising a more rigid oligomerisation system [44] (Fig. 1).

Drp1, cardiolipin and reactive oxygen species

The mechanistic interplay between Drp1 and mitochondrial phospholipids has been a topic of intense research recently. This originates from the fact that the activity of yeast Drp1 was shown to be stimulated in the presence of liposomes that mimic the mitochondrial outer membrane (MOM) [46]. Further research into identifying individual phospholipids important for this stimulation reveals cardiolipin (CL) as the most potent stimulator of Drp1 GTPase activity [59, 60]. CL is a mitochondrial-specific phospholipid that contributes 12–17 % of the total mitochondrial phospholipid content, present largely in the inner membrane (14–23 %) and a smaller proportion in the outer membrane (3–10 %) [61]. Conical-shaped CL has a non-bilayer forming propensity and may facilitate the formation of an intermediate state during membrane remodelling events including fission and fusion [62–65]. A direct interaction between Drp1 and CL-containing membranes was observed in vitro using a Trp-Dansyl FRET assay [60] whereas lipid dot-blot analysis found that direct Drp1–CL interactions were mediated by four lysine residues in the InsB region of Drp1 that are conserved among vertebrates [59] Disruption of an essential cardiolipin synthase gene (*cls-1)* in *Arabidopsis thaliana* leads to a block in fission resulting in unbiased fusion [64]. The block in fission is attributed to the loss of the Drp3 (the plant Drp1 ortholog) complex at mitochondria which is stabilised by cardiolipin thus highlighting the functional consequence of the interaction in vivo [64]. Thus a growing body of research lays emphasis on the Drp1–CL interactions as an important step for Drp1-dependent mitochondrial fission.

One of the factors that can affect the mechanism of Drp1-induced mitochondrial fragmentation from within mitochondria is reactive oxygen species (ROS). ROS are damaging by-products of the electron transport chain (ETC), housed with the mitochondrial inner membrane (MIM). ROS are initially formed by the premature release of electrons from the ETC and the reduction of molecular oxygen to form the superoxide radical $^{\cdot}O_2^-$ which can damage mitochondrial components including mtDNA, respiratory chain complexes and lipid membranes [66, 67]. Increases in mitochondrial fragmentation and ROS have been observed in many disease states including diabetes, ischaemia reperfusion injury (IRI), AD and PD [68–72]. In IRI, the production of ROS in the reperfusion stage results in fragmentation of the mitochondrial network [73]. Interestingly, inhibiting mitochondrial fission or promoting fusion in a HL-1 model can attenuate the onset on IRI and delay cell death, suggesting other complex factors are at play [73]. Although mitochondria house proteins are capable of detoxifying superoxide (such as manganese superoxide dismutase), excessive levels of ROS can overwhelm such systems. In order to reduce the levels of ROS, exogenous antioxidants have been employed to minimise the lipid peroxidation induced by ROS [74, 75]. Studies in both cell and animal models for PD, AD, cardiac disease, metabolic syndrome and type I diabetes suggest that treatment with MitoQ, a lipophilic ubiquinone molecule that selectively accumulates within mitochondria in vivo, can protect against oxidative damage and the downstream effects that follow [69, 76–79]. Although often seen occurring concomitantly in disease models such as IRI, the relationship between ROS and mitochondrial fission is not well understood. Superoxide is produced at seven different sites associated with proteins across the MIM, yet only two of these sites release superoxide into the IMS [80]. Although these superoxide molecules will directly damage lipids of either the inner leaflet of the MOM or the MIM, exactly how lipid peroxidation triggers Drp1 recruitment and subsequent fission remains unknown.

Adaptor proteins for Drp1/Dnm1 recruitment and fission

Fis1 and Mdv1/Caf4

Yeast screens identified Fis1 as the first integral membrane protein to play a role in mitochondrial fission [81, 82]. Fis1

is C-terminally tethered to the mitochondrial outer membrane and contains an α-helix, involved in oligomerisation, followed by two tetratrico-peptide repeat (TPR) motifs [83]. This well-defined protein–protein interaction motif is commonly associated in multiprotein complexes and comprises helix–turn–helix repeats arranged in an antiparallel fashion [84]. Fis1 is also expressed on peroxisomes and has been shown, along with Drp1, to mediate fission of peroxisomal membranes [85–87]. In yeast, Fis1 forms a central component of the mitochondrial dynamin adaptor complex, where it stabilises the peripheral membrane proteins Mdv1 and Caf4 to the mitochondrial outer membrane, which in turn recruit Dnm1 to mitochondria [81, 88–90]. Mdv1 and Caf4 are orthologous proteins with WD40 repeat β-propeller motifs that form foci with Dnm1. Crystal structures revealed that the αA and αB helices of both Mdv1 and Caf4 pack into a concave hydrophobic groove on Fis1 (Fig. 2) [82, 91]. Furthermore, the structure of the Fis1–Mdv1 complex, including the Mdv1 coiled-coil region, reveals formation of a compact dimer stabilised by a scaffolding contact between Fis1 and the Mdv1 coiled-coil, which would position the β-propeller toward the cytosol. It was recently shown that yeast Fis1 is dispensable for recruitment of Dnm1 for fission when Mdv1 is tethered to the membrane, which indicates that the role of Fis1 in Dnm1 recruitment may simply present a protein fold for docking Dnm1 adaptors onto the mitochondrial surface [92]. Interestingly, the Mdv1–Dnm1 interaction is mediated through a number of critical residues on the WD40 repeat and InsB presenting the first described function of InsB in a dynamin-related protein–adaptor interaction [51].

Despite its evolutionary conservation, the role of Fis1 in mammalian mitochondrial fission remains controversial. Early work indicated that overexpression of human Fis1 results in mitochondrial fragmentation with loss inducing mitochondrial elongation [93–95]. More recently, however, deletion of Fis1 in mouse embryonic fibroblasts indicated only a minor role in mitochondrial fission with no obvious differences in mitochondrial morphology or fusion rates observed [47, 96]. Also, the lack of Fis1 did not affect Drp1's association with mitochondria [16, 36, 47]. Recent co-immunoprecipitation studies have suggested that Fis1 forms part of a larger fission complex at the ER–mitochondrial interface, containing the Mitochondrion-Associated Membrane (MAM) ER proteins Bap31 and Calnexin [97, 98]. Fis1 has also been suggested to mediate stress-induced mitochondrial fission as part of an apoptotic response [99]. Further to this it has also been proposed that Fis1 may act in a fission sequence after Drp1 and other adaptors—perhaps in relation to a stress response rather than normal cell homeostasis [98]. Depending on the stimulus, Fis1 may contribute to division of mitochondria in the apoptotic or mitophagy pathway [98]. The lack of mammalian homologs of Mdv1 and Caf4 coupled to the increasing evidence that Fis1 is not the primary mammalian mitochondrial adaptor for fission suggests an evolutionary divergence in the fission process.

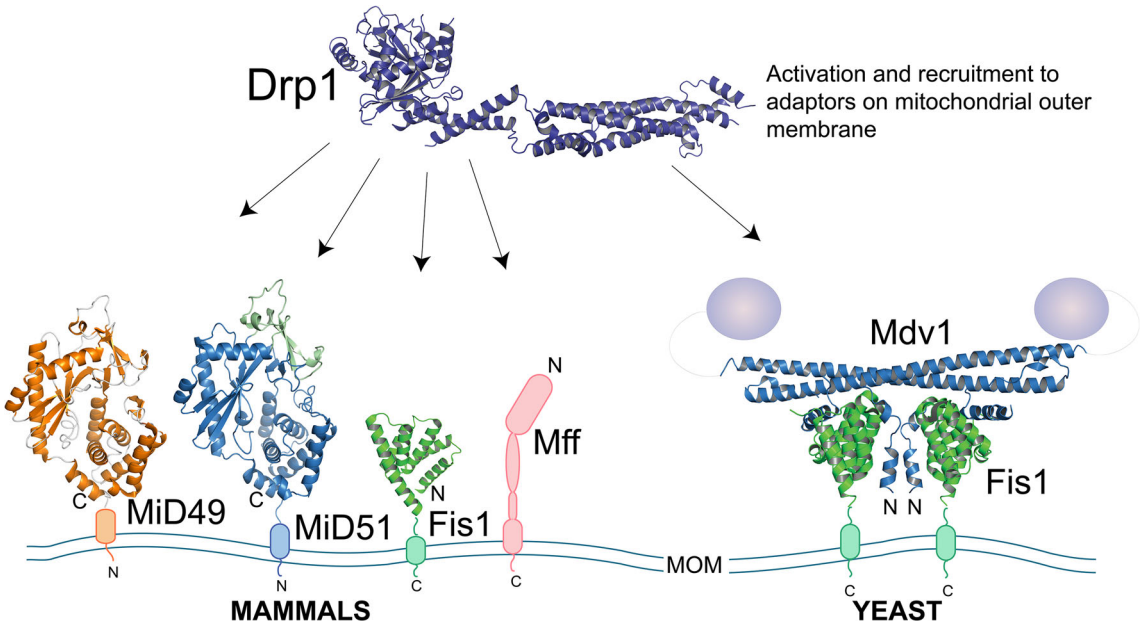

Fig. 2 Mitochondrial division in mammals and yeast. In mammals, Drp1 is recruited to the mitochondrial outer membrane by adaptors MiD49, MiD51, Mff and possibly Fis1 (PDB accession numbers: Drp1: 4BEJ; MiD49: 4WOY, MiD51: 4NXV, Fis1: 1NZN). Division in yeast is controlled by Dnm1, which is recruited by the β-propeller domain of Mdv1, and stabilised in a dimer by the TPR domain of Fis1 at the outer mitochondrial membrane (PDB accession numbers: Fis1–Mdv1 complex, 3UUX)

Mitochondrial fission factor, Mff

Mitochondrial fission factor (Mff) was the first mitochondrial adaptor shown to recruit Drp1 to mitochondria in a Fis1-independent manner [47, 85]. siRNA knockdown of Mff inhibited mitochondrial fission and delayed cytochrome *c* release during apoptosis [85]. Peroxisomal fission was also inhibited upon Mff depletion resulting in peroxisomal extensions [47, 85], indicating that Mff, like Drp1, is involved in both mitochondrial and peroxisomal fissions. Indeed, an interaction with the peroxisomal fission mediator Pex11p has been demonstrated by co-immunoprecipitation and was shown to require Drp1 [100, 101]. Mff localises to discrete foci on mitochondria and co-localises with Drp1 [47, 85]. It was shown that Mff-induced fission requires the presence of Drp1, and that Drp1 association with mitochondria is reduced in Mff-depleted cells [47]. Mff and Drp1 co-immunoprecipitate; however, the requirement for chemical crosslinking indicates a transient interaction [47]. It now appears that the Mff is a more potent mediator of mitochondrial fission than Fis1, as the presence of Mff and Drp1, in contrast to Fis1 and Drp1, was found to be sufficient to produce fission in a yeast system devoid of its own fission mediators (Dnm1, Fis1, Mdv1) [92]. In keeping with this, reintroduction of Mff in MEFs lacking endogenous Mff or Fis1, elicited a pronounced Drp1 recruitment to mitochondria [96].

Mff is C-terminally anchored to the mitochondrial outer membrane and contains two conserved short N-terminal amino acid repeats, suggested to be binding motifs (Fig. 2) [85]. It also contains a predicted coiled-coil domain, which is required for correct targeting to mitochondria. Investigation of the Mff–Drp1 interaction using BRET, co-immunoprecipitation and functional assays revealed a binding interface on Drp1 containing the highly conserved R376 of the stalk domain [50]. The interaction of Mff and Drp1 at this site is proposed to contribute to the spiral compaction of Drp1 oligomers on the mitochondrial outer membrane [50]. The involvement of Mff in fission in higher eukaryotes provides an intriguing starting point for the elucidation of complex assembly; however, specific binding mechanisms remain to be demonstrated with structural information.

Mitochondrial dynamics proteins MiD49 and MiD51

Mitochondrial dynamics proteins of 49 and 51 kDa (MiD49 and MiD51) are mitochondrial outer membrane proteins specifically found in chordates. MiD51 was identified as part of a random cellular localisation screen of uncharacterised human proteins, which following overexpression, resulted in changes in mitochondrial dynamics [102]. MiD49 was subsequently identified due to sequence homology [103]. MiD49 and MiD51 share 45 % sequence identity yet lack significant sequence homology to other proteins. In contrast to Fis1 and Mff, MiD49 and MiD51 appear to be exclusively mitochondrial, indicating a mechanism to ensure specificity and selectivity of Drp1-mediated fission at mitochondria [103]. MiD49 and MiD51 are N-terminally tethered to the mitochondrial outer membrane and consist of a transmembrane domain, a predicted disordered region of approximately 70 amino acids followed by a cytosolic domain (Fig. 2). Confocal microscopy analysis demonstrated the localisation of MiD51 seems to occur around mitochondrial constriction sites, much like Drp1 itself [103]. In cells overexpressing MiD49 or MiD51, the predominantly cytosolic Drp1 was enriched at mitochondria at sites containing MiD49/51-GFP [103]. Using yeast two-hybrid assays and co-immunoprecipitation/cross-linking, a direct interaction between Drp1 and MiD proteins was observed [92, 96, 103, 104]. Overexpression of MiD49/51 produces two distinct mitochondrial morphology states depending on MiD protein levels—either forming elongated tubules that project from a perinuclear collapsed network (high level expression), or a relatively normal morphology accompanied by MiD49/51 foci formation (low level expression). In the context of a role as fission mediators, elongation of mitochondrial tubules upon overexpression of MiD49/51 appears counterintuitive. However, given that the high levels of overexpressed protein far exceed physiological levels, it was proposed that the phenotype is a result of sequestration of Drp1, rendering the GTPase non-functional [103]. The observation was contradictorily interpreted as a role of MiD51 (independently identified as MIEF1) in the promotion of mitochondrial fusion rather than fission [105]. However, studies from other laboratories have confirmed that MiD49/51 mediate Drp1-driven mitochondrial fission [92, 96]. MiD49 and MiD51 were able to restore CCCP-induced mitochondrial fragmentation in Fis1/Mff-knockout cells and their knockdown was independently shown to cause elongation [96]. Furthermore, induction of MiD49/51 expression caused mitochondrial elongation through Drp1 inactivation [104], while MiD49 with Drp1 could drive fission events in a yeast line lacking endogenous fission machinery [92]. Interestingly, in vitro, addition of MiD49 was found to decrease the external diameters of Drp1-constricted liposomes from 31 to 15 nm, a distance amenable to membrane scission in the context of the mitochondrial double membrane [92]. This indicates that MiD49/51 may function downstream of Mff and that they are actively involved in facilitating Drp1-mediated mitochondrial scission.

The lack of MiD49/51 sequence homology to known protein structures or obvious structural motifs has made deduction of functional domains or a protein-binding site

difficult. Recently, the MiD51 cytosolic domain was independently solved by two groups and found to harbour a nucleotidyltransferase fold (Fig. 2) [106, 107]. However, MiD51 lacks the key catalytic triad of residues that define a protein as a *bone fide* nucleotidyltransferase. The distal cytosolic loop of MiD51 contains a region of the protein responsible for the interaction with Drp1, termed the Drp1 recruitment region (DRR). Deletion of this loop, five residues in length, does not impair the overall fold of the protein, but does block the ability of MiD51 to recruit Drp1 to mitochondria [107]. Interestingly, although MiD51 can bind ADP and GDP in the fold, mutations that disrupt nucleotide binding in this region did not abrogate recruitment of Drp1, indicating adaptation of the nucleotidyltransferase fold for a novel purpose [107]. However, Chan and colleagues demonstrated that binding ADP promotes Drp1 assembly into spirals and enhances GTP hydrolysis—leading to the fact that ADP is a co-factor for MiD51-dependent Drp1 fission [108]. Structural analysis of MiD49, like MiD51, revealed it also harbours a nucleotidyltransferase fold; however, MiD49 does bind nucleotides like in homolog MiD51 (Fig. 2) [109]. Further to this, the crucial residues needed to bind nucleotides are not conserved, suggesting functional disparity between the two proteins. However, the Drp1 surface-binding loop is conserved between the two proteins [109]. Although the physiological consequences of MiD51-binding dinucleotide diphosphates and MiD49 not binding are unclear, it has provided a starting point for determining the mechanistic contribution of the MiD proteins in mitochondrial fission.

The role of fission in apoptosis and mitophagy

Tissue and organ maintenance requires elimination of dysfunctional cells though apoptosis. By housing and releasing pro-apoptotic molecules such as a cytochrome *c*, mitochondria are central to the regulation of the intrinsic apoptotic pathway [4]. Fragmentation of the mitochondrial network is often seen in apoptosis and precedes caspase activation, mitochondrial outer membrane permeabilization, cristae remodelling (such as Opa1 processing) and cytochrome *c* release [110]. Central to this is the interaction of proteins belonging to the Bcl-2 family (which regulate apoptotic progression) and morphology proteins—these interactions have been observed during the progression of programmed cell death [93, 111–113]. Under normal homeostasis, Mfn1 and Mfn2 interact with Bak and VDAC2 at MOM [111, 114]. The interaction differs in apoptotic cells where Bak becomes dissociated from Mfn2 and VDAC2, but remains in complex with Mfn1 [111]. At the MOM, Drp1 interacts with Bak and translocated Bax, where it becomes sumoylated [36]. This modification results in a stable association of Drp1 with the MOM. The role of modified Drp1 in apoptotic fission, however, is not well understood, as a mutant of Drp1 (that is unable to be sumoylated) can still be recruited to the MOM following apoptotic stimuli, suggesting that sumoylation is not a requirement/or specific to apoptotic fission [36]. Further to this, a role for Drp1 in Bax oligomersiation, subsequent pore formation and cytochrome *c* release has been suggested [115]. Independent of its role as a GTPase, Drp1 has been shown localised to areas rich in CL in artificial membranes [115]. These regions containing Drp1 oligomers and CL appear to be a hot spot for membrane tethering and hemifusion in vitro [115]. These events may allow Bax oligomerisation and pore formation crucial for cytochrome *c* release and subsequent caspase activation. If indeed Drp1 does play such a pivotal role in the progression of apoptosis, this may explain the delay in programmed cell death and reduction in cytochrome *c* release when Drp1 is inhibited or deleted [30, 31, 116, 117].

Like apoptosis, mitophagy is a selective process—removing damaged mitochondria from the cell to prevent death on a larger scale. Modulation of mitochondrial morphology is one of the many important steps in mitophagy as fragmented mitochondria are often (but not always) observed prior to mitophagy [22]. In keeping with this, many studies have linked the rate of mitophagy to the size of mitochondria. Mitochondria visualised within autophagosomes have been reported to be <1 μm in diameter [22, 118–120]. However, this is not seen exclusively, making it complex to delineate. Both overexpression of Opa1 and inhibition of fission in a pancreatic β-cell line result in a ~ 70 % reduction in mitophagy rates [22]. These conflicting results suggest that organelle length may not be a rate-limiting step in mitophagy. Fis1 has recently been implicated in the disposal of defective mitochondria [98, 121]. Although once thought to be the main MOM adaptor of Drp1, it now appears that Fis1 plays a key role in stress-induced fission, as it can be immunoprecipitated with Drp1 when fission is chemically induced [98]. Mutations or knockout of Fis1 in *C. elegans* or mammalian cells show increased amounts of LGG-1/LC3 aggregates, suggesting that Fis1 augments the removal of these aggregates in times of cellular stress [98].

The involvement of both division and fusion prior to mitophagy highlights the importance of mitochondrial homeostasis in cellular health. In contrast to fission, fusion requires an intact membrane potential ($\Delta\Psi_m$) making this a selective process and excluding mitochondria that are deemed 'unfit'. Termed 'kiss-and-run' events, a brief fusion event is followed by division of the mitochondrion. These fusion/fission events often result in daughter mitochondria with unequal $\Delta\Psi_m$ [22]. If the $\Delta\Psi_m$ is significantly low in one of these mitochondria, PINK1 is

unable to be imported into mitochondria and remains at the MOM for signalling; as such PINK1 is often referred to as a sensor of mitochondrial fitness [122]. In keeping with this role as a sensor, outer membrane accumulated PINK1 phosphorylates ubiquitin directly, which in turn activates the E3 ubiquitin ligase activity of Parkin [123–125]. Activation and recruitment of Parkin allows autophagosome adaptors to assemble, recruit and help form the mature autophagosome [126–128]. This process results in degradation of the daughter mitochondrion with a lower $\Delta\Psi_m$ [22]. However, if the mitochondrion can recover its $\Delta\Psi_m$ before the recruitment of Parkin, it can re-enter the fission/fusion cycle [22, 129]. This suggests that mitochondrial fission, which is often a cellular response to stress, is a mechanism utilised by the cell to segregate and eliminate damaged mitochondria from an otherwise healthy network.

ER–mitochondrial interactions in fission

Mitochondria and the endoplasmic reticulum are engaged in a close spatial relationship that mediates Ca^{2+}, apoptotic signalling and lipid metabolism between membranes [130, 131]. The close contacts these organelle networks make has been measured by electron tomography (ET) to be 9–30 nm wide and to usually occur in clusters, allowing bidirectional trafficking of factors across membranes as well as communication between organelles [132]. More recently, evidence has revealed links between ER–mitochondrial contacts with mitochondrial fission [133] (Fig. 3). Electron microscopy and tomography were used to visualise ER–mitochondrial contacts in yeast, and demonstrated that the ER forms rings around mitochondria, where tubule constriction sites with smaller diameters were bordered by a higher percentage of ER wrapping [133]. The study also showed that Drp1-mediated mitochondrial division in mammalian cells occurs at ER–mitochondrial contact sites and that this contact precedes interaction with Drp1 and Mff. This marking of fission sites by the ER suggests that ER proteins may be closely linked to fission or that the ER presents a physical constriction element, perhaps crudely reshaping the mitochondrial tubule before fission adaptors and effectors assemble and execute division [131].

Two recently characterised proteins have been identified to mediate the ER–mitochondrial connections related to fission. INF2 (inverted formin 2) was originally identified as a protein capable of bundling microtubules and interacting with actin, INF2 is an ER protein that drives actin polymerisation (in conjunction with myosin II) and stimulates Drp1 at fission sites [134–136]. The myosin link is intriguing as it accumulates on mitochondria in an actin/INF2-dependent manner. Further to this, inhibition of myosin 2 with blebbistatin, decreases the Drp1 association with mitochondria [135]. Taken together, these results suggest an important mechanistic role in which actin polymerisation, mediated by INF2 leads to myosin II recruitment and constriction of mitochondria at the fission site, thus enhancing Drp1 accumulation and subsequent fission. An ER-associated soluble *N*-ethylmaleimide-

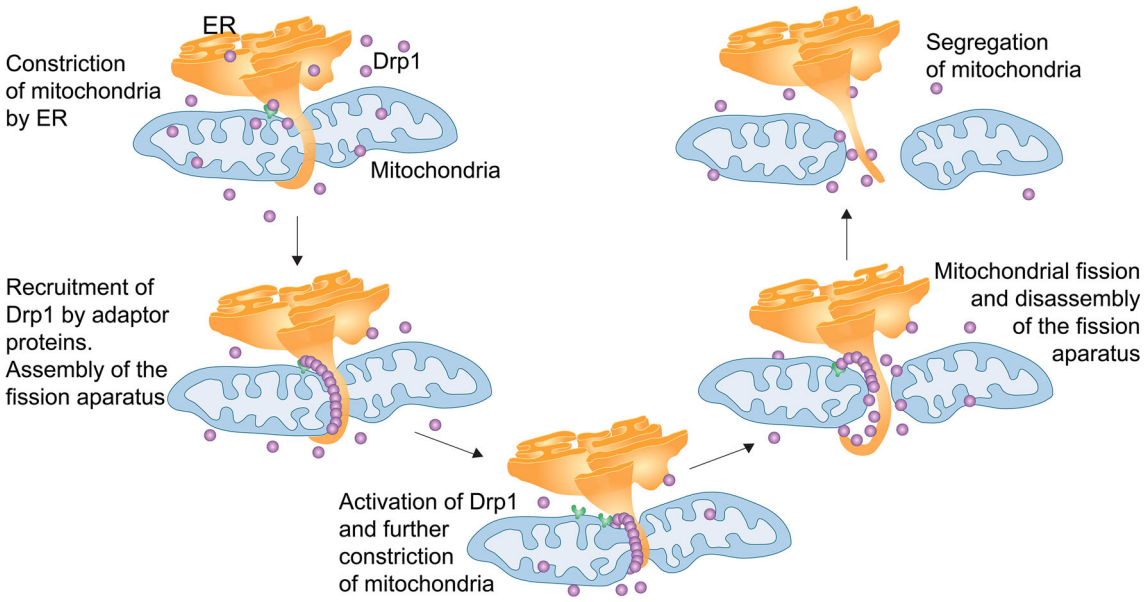

Fig. 3 ER–mitochondrial contacts in mitochondrial division. The ER forms extensions around mitochondria and physically constricts the organelle. Adaptor proteins such as MiD49/51 and Mff recruit cytosolic Drp1 to this scission site, where the organelle is constricted further. The fission apparatus is assembled and division of mitochondria occurs

sensitive factor attachment protein (SNAP) receptor (SNARE) protein syntaxin 17 (Syn17) was also recently found to direct Drp1 to these contact sites (by binding specifically to Mff), thus promoting Drp1-driven division of mitochondria [137]. This suggests that the Syn17–Mff complex may be involved in the initial assembly of Drp1 at the constriction site. In order to regulate this direction of Drp1 to contact sites, Syn17 also interacts with Rab32, a mitochondrial-associated membrane-localised A-kinase anchor protein. Rab32 has been shown to determine targeting of PKA to the mitochondrial and ER membranes, phosphorylating Drp1 at S637, resulting in a block in the fission process [138]. This interaction between Syn17 and Rab32 appears to be a regulatory checkpoint as the Syn17/Rab32 interaction prevents post-translational modification of Drp1, while depletion of Syn17 increases phosphorylation [137]. Upon starvation conditions, Syn17 preferentially binds ATG14L over Rab32 and Drp1, causing mitochondrial elongation in the initial phase of autophagy [137]. This mechanism is particularly intriguing as it sheds light on how the ER tubule is directed to the future fission site on mitochondria—through the Syn17–Mff complex.

Concluding remarks

Regulation of mitochondrial fission is a complex process, made more convoluted by the fact that there are clear differences in the machineries between yeast and animals. There are many proposed mitochondrial Drp1 adaptors—how exactly these function together (and how they co-ordinate the scission process with fission mediators of the inner membrane) remains to be clarified. A possibility is that divergent regulators are required for different aspects of fission and/or tissue specific fission regulators. For example, while Fis1 and Mff have a dual mitochondrial and peroxisomal location, MiD49 and MiD51 appear to be specific mitochondrial fission factors [47, 87, 104]. Fis1 once thought to be integral to Drp1 recruitment in both yeast and mammalian mitochondria now appears to have a highly specialised role in fission [34, 98]. While Mff is largely ubiquitously expressed, MiD49 and MiD51 display differential tissue expression.

The future challenge to understand the precise mechanistic detail into the assembly of the mitochondrial fission apparatus revolves around cementing the roles of adaptor proteins along with the involvement of the cytoskeleton and ER in executing membrane scission with Drp1. The exact triggers for productive assembly of the fission apparatus are not yet clear, nor is the involvement of the inner membrane and correct positioning of mtDNA [20, 139]. A better understanding of lipid remodelling/composition at the site of scission is also required to help deduce how fission is executed.

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