© 2017. Published by The Company of Biologists Ltd | Journal of Cell Science (2017) 130, 2953-2960 doi:10.1242/jcs.199562

REVIEW

The constriction and scission machineries involved in mitochondrial fission

Felix Kraus and Michael T. Ryan*

ABSTRACT

A key event in the evolution of eukaryotic cells was the engulfment of an aerobic bacterium by a larger anaerobic archaebacterium, leading to a close relationship between the host and the newly formed endosymbiont. Mitochondria, originating from this event, have evolved to be the main place of cellular ATP production. Maintaining elements of their independence, mitochondria undergo growth and division in the cell, thereby ensuring that new daughter cells inherit a mitochondrial complement. Mitochondrial division is also important for other processes, including quality control, mitochondrial (mt)DNA inheritance, transport and cell death. However, unlike bacterial fission, which uses a dynamin-related protein to constrict the membrane at its inner face, mitochondria use dynamin and dynamin-related proteins to constrict the outer membrane from the cytosolic face. In this Review, we summarize the role of proteins from the dynamin superfamily in mitochondrial division. This includes recent findings highlighting that dynamin-2 (Dnm2) is involved in mitochondrial scission, which led to the reappraisal of the role of dynamin-related protein 1 (Drp1; also known as Dnm1l) and its outer membrane adaptors as components of the mitochondrial constriction machinery along with ER components and actin.

KEY WORDS: Drp1, Mff, MiD51, Dynamin, Mitochondrial dynamics, Mitochondrial fission

Introduction

It has been well documented that the mitochondrial network in cells undergoes both fission and fusion events (Friedman and Nunnari, 2014; Lackner, 2014; Richter et al., 2015). Fusion is important to mix mitochondrial contents to maintain a homogeneous population of organelles, but what of fission? A logical reasoning is that fission is required to ensure that a population of organelles is available for segregation and inheritance by daughter cells during mitosis. Evidence for this is available through the knockout of the fission mediator dynamin-related protein 1 (Drp1; also known as Dnm1L), which leads to entirely connected organelles, sometimes causing cells to display cytokinesis defects (Ishihara et al., 2009). However, cells that are blocked in mitochondrial fission and contain a highly fused network can still grow and divide in culture, so fission is not essential for cell viability. Furthermore, not all cells divide (e.g. neurons) and yet their mitochondria still undergo fission, pointing to additional functional roles. An alternative role for fission is to produce smaller organelles that are simply more easy to transport along cytoskeletal tracks in cells – for example in neurons with long and thin axonal processes. Indeed, mice lacking mitochondrial fission specifically in neurons die shortly after birth (Ishihara et al., 2009). An additional role for fission is in quality control to maintain a healthy population of organelles. It was found that mitochondrial division under normal cell growth conditions can result in the generation of daughter mitochondria with unequal fitness, and this may allow for selective mitophagy of the more defective organelle to occur (Twig et al., 2008). Smaller mitochondria, which are generated through fission, also allow for more efficient engulfment by the autophagosomal machinery, while interconnected mitochondria are protected (Gomes et al., 2011; Rambold et al., 2011). Defects in fission can also lead to increased oxidative damage and neuronal cell death (Kageyama et al., 2012). In addition to these roles, mitochondrial fission often occurs after apoptotic induction to facilitate the release of cytochrome *c*. While mitochondrial fission is not essential for apoptosis, blocking fission leads to a delay in cytochrome *c* release from mitochondria, even when the pro-apoptotic proteins Bax and Bak (also known as Bak1) have been activated (Martinou and Youle, 2011; Otera et al., 2016). It is thought that mitochondrial fission somehow aids in the remodelling and opening of cristae tubules that sequester the bulk of cytochrome *c* (Pernas and Scorrano, 2016). More recently, mitochondrial fission has been shown to be linked to the replication and transfer of mitochondrial (mt)DNA between organelles (Lewis et al., 2016). In particular, it was found that mtDNA, which localizes close to the inner face of the mitochondrial inner membrane, is often present at mitochondrial constriction sites where the fission machinery is assembled. Moreover, new copies of mtDNA are made at sites that are destined for fission so that daughter mitochondria can inherit their own complement of mtDNA (Lewis et al., 2016).

Mitochondrial dynamics is also linked to the metabolic state of the cell (Buck et al., 2016; Mishra and Chan, 2016; Roy et al., 2015). Mitochondria with higher respiration rates have been reported to be more elongated, whereas non-respiring mitochondria become fragmented through fission (Plecita-Hlavata et al., 2008). Finally, one might expect that a tangled network of mitochondria may not be entirely conducive to proper cellular function. By reducing mitochondrial size through fission, this may help to provide order to the cell. Indeed, contacts made between the endoplasmic reticulum (ER) tubules and mitochondria facilitate fission (Prudent and McBride, 2017), and this may help to prevent entanglement between these two membranous organelles. In this Review, we discuss the function of dynamin superfamily members as drivers of mitochondrial fission. With the recent discovery that dynamin-2 (Dnm2) has a role in the division of mitochondria, an updated model of fission is presented and core cellular components aiding the process are discussed.

Drp1 and its adaptors

Membrane fission is required for the function of many cellular processes. It is fundamentally a stochastic process, and hence can

Department of Biochemistry and Molecular Biology, Monash Biomedicine Discovery Institute, Monash University, 3800 Melbourne, Australia.

*Author for correspondence (michael.ryan@monash.edu)

F.K., 0000-0002-3757-541X; M.T.R., 0000-0003-2586-8829

Journal of Cell Science (2017) 130, 2953-2960 doi:10.1242/jcs.199562

only occur if the energetic state of fission is more favourable than that of not undergoing fission (Antonny et al., 2016; Mattila et al., 2015; Merrifield et al., 2005; Sundborger et al., 2014). Consequently, fission is aided by membrane constriction machineries that reduce the energy barrier between those two states, leading to enhanced probabilities of membrane fission (Morlot et al., 2012). Dynamin GTPases are involved in polymerizing around and constricting a variety of membranes to drive fission (Fig. 1A). One of the core proteins well known to be involved in mitochondrial fission is Drp1 (Dnm1 in yeast) (Smirnova et al., 2001). This ∼80 kDa GTPase is predominantly cytosolic and is recruited to the mitochondrial outer membrane to drive fission (Smirnova et al., 2001). Drp1 is also essential for peroxisomal fission, and loss of Drp1 results in highly elongated mitochondria and peroxisomes (Ishihara et al., 2009; Koch et al., 2003; Wakabayashi et al., 2009). Drp1 shows structural similarity to dynamin proteins (Fig. 1B), but there are critical differences that affect its membrane assembly and constriction potential (Fröhlich et al., 2013). Like dynamins, Drp1 monomers oligomerize and form contractile rings (Fröhlich et al., 2013; Ingerman et al., 2005; Korobova et al., 2013; Mears et al., 2011). Following GTP hydrolysis, conformational changes in Drp1 helices cause a two-fold decrease in ring diameter (Koirala et al., 2013; Mears et al., 2011), which facilitates membrane constriction. The dynamin spirals assemble around a membrane and nucleate from one starting point, forming a one-start helix (Zhang and Hinshaw, 2001). In contrast, Drp1 spirals nucleate around the membrane from two adjacent starting points and hence form a two-start helix (Fröhlich et al., 2013). Assembly is mediated through the central stalk interface in an X-shaped fashion (Fig. 1C) (Ford et al., 2011; Fröhlich et al., 2013).

In contrast to classical dynamins, Drp1 does not contain a phospholipid-binding pleckstrin homology (PH) domain, raising the question of how the protein is recruited to and assembled at membranes. Currently, a number of different mitochondrial receptors and/or adaptors for Drp1 have been characterized. In yeast, Fis1 acts as a mitochondrial outer membrane adaptor for the yeast-specific peripheral membrane receptors Mdv1 and Caf4, which then recruit and assemble with Dnm1 (Lackner et al., 2009). In metazoans, there are no homologs for Mdv1 or Caf4, and it has now been established that Fis1 is not required for fission (Osellame et al., 2016; Otera et al., 2010). Instead, mitochondrial fission factor (Mff) along with mitochondrial dynamics proteins of 49 and 51 kDa (MiD49 and MiD51; encoded by *MIEF2* and *MIEF1*, respectively), which are anchored in the outer mitochondrial membrane, bind to and orchestrate the assembly of the contractile Drp1 rings at constriction sites (Gandre-Babbe and van der Bliek, 2008; Losón et al., 2013; Otera et al., 2010; Palmer et al., 2013, 2011). Mff appears to be the primary adaptor protein as it is present in all metazoans and is involved in the recruitment of Drp1 to both mitochondrial and peroxisomal membranes (Gandre-Babbe and van der Bliek, 2008; Losón et al., 2013; Otera et al., 2010). Accordingly, knockout cell lines of Mff show highly elongated mitochondria and peroxisomes (Chen et al., 2015; Losón et al., 2013; Osellame et al., 2016; Otera et al., 2010). MiD49 and MiD51 are chordate-specific mitochondrial outer membrane proteins (Palmer et al., 2011). They have inactive nucleotidyltransferase folds and can recruit Drp1 to mitochondrial fission sites independently of Mff (Losón et al., 2014, 2015, 2013; Osellame et al., 2016; Otera et al., 2016; Richter et al., 2014). In the absence of Drp1, MiD49 and MiD51 become diffusely located on the mitochondrial outer membrane, whereas, in contrast, Mff remains in foci associated with constriction sites (Friedman et al., 2011; Otera et al., 2010; Richter et al., 2014).

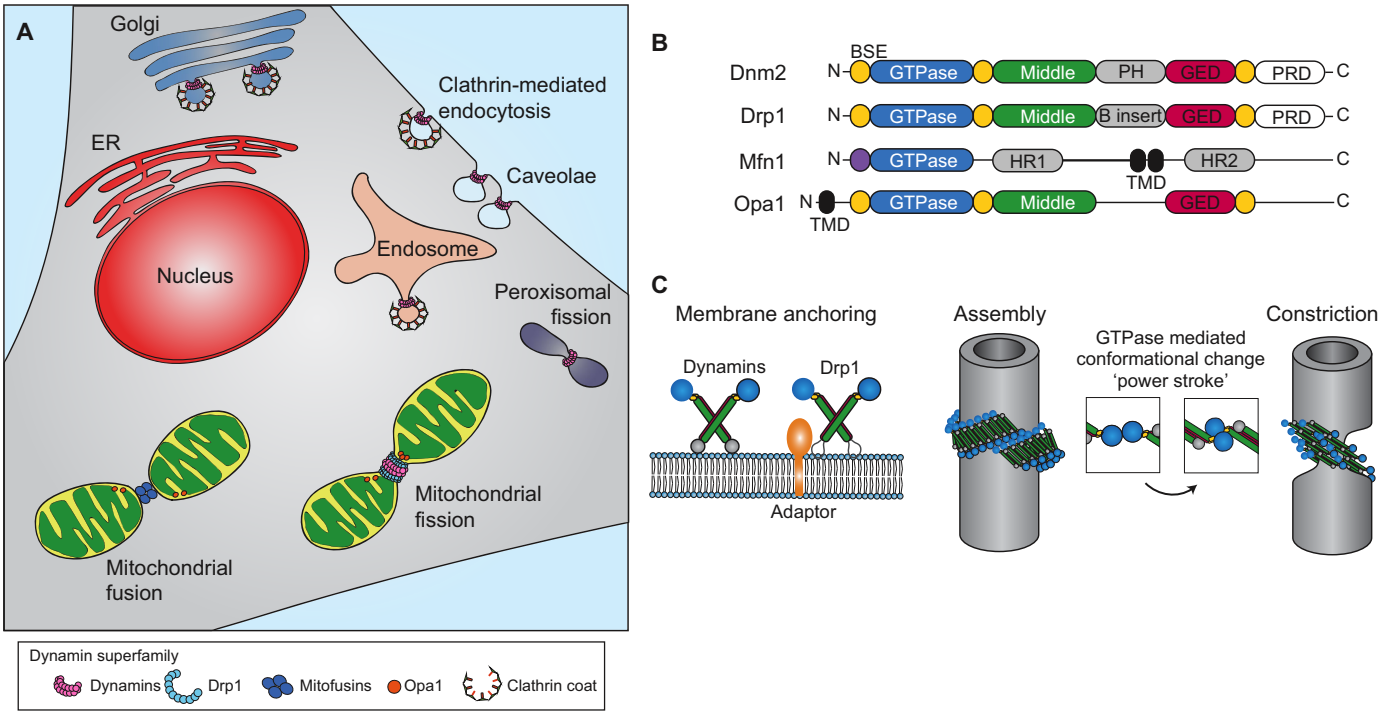

Fig. 1. Functional roles of dynamin-superfamily member proteins. (A) Cellular localization of dynamin superfamily proteins. Dynamin family members are involved in various cellular processes including organelle dynamics and vesicle formation in the endocytic and secretory pathways. (B) Schematic representation of the protein domain organization of key dynamin family members involved in mitochondrial dynamics. (C) Mechanism of dynamin-mediated membrane tubule constriction. Left panel, membrane association of dynamin and Drp1 using colour coding from B. Right panel, dynamin scaffold assembly and membrane tubule constriction mediated by the conformational change of dynamins.

Although Mff and MiD49 and/or MiD51 can act independently in recruiting Drp1 to mitochondria for fission, they appear to differentially modulate Drp1 function. Mff bound to liposomes can stimulate the GTPase activity of Drp1, whereas, in contrast, MiD51 inhibits this activity (Macdonald et al., 2015; Osellame et al., 2016). Furthermore, Drp1-mediated fission through MiD49 and/or MiD51, but not Mff, is required for cristae opening and cytochrome *c* release following apoptotic induction, indicating that distinct fission mechanisms may exist (Otera et al., 2016). MiD49 and MiD51 may have a similar function to the yeast Mdv1 adaptor, as they all can assemble with Drp1/Dnm1 spirals, potentially facilitating membrane constriction (Lackner et al., 2009; Naylor et al., 2006). Indeed, *in vitro* measurements of lipid tubules has shown that MiD49 increases the ability of Drp1 to constrict the tubules, giving rise to MiD49-Drp1 rings of ∼15 nm in diameter (Koirala et al., 2013). Nevertheless, Mff and MiD51 have been found together in proximity with Drp1 in cells (Osellame et al., 2016).

Dnm2 in mitochondrial fission

During division, the adaptors remain with the assembled Drp1-mediated constriction rings, and after division, a population of Drp1 together with the adaptors (at least MiD49 and MiD51, and Mdv1) is found on each organelle (Lackner et al., 2009; Legesse-Miller et al., 2003; Naylor et al., 2006; Richter et al., 2014). The physical separation of two populations of assembled Drp1 polymers suggests that membrane scission occurs towards the centre of the constriction site. While it had been assumed that the final membrane scission events were achieved by Drp1-induced constriction forces, Voeltz and colleagues have now demonstrated that a member of the conventional dynamin family, Dnm2, is responsible for these processes (Lee et al., 2016). Knockdown of Dnm2 led to elongated mitochondria with the occasional presence of a long, highly constricted tubule between two populations of preassembled Drp1 polymers. In contrast to Drp1, which can be found on most constricted mitochondrial sites, Dnm2 only localizes transiently to facilitate membrane scission. Moreover, fluorescently labelled Dnm2 and Drp1 are differentially segregated to daughter organelles – Drp1 remains present on both daughter mitochondria following scission, whereas Dnm2 appears only on one of the two (Lee et al., 2016). This study thus sheds new light into the cooperative nature of the dynamin superfamily and the ability of dynamins to coordinate membrane scission in a sequential manner. These results also explain some of the earlier *in vitro* studies assessing Drp1 constriction, which described that Drp1 can readily tubulate liposomes, but not sever them (Yoon et al., 2001). Of note, it has been shown that dynamin-1, which is closely related to Dnm2, has the ability to drive fission of liposomes *in vitro* (Sweitzer and Hinshaw, 1998).

This new finding for the role of Dnm2 in mitochondrial dynamics also has implications for disease pathogenesis (Durieux et al., 2010). Mutations in Dnm2 cause Charcot–Marie–Tooth neuropathy (Züchner et al., 2005), as well as centronuclear myopathy (Bitoun et al., 2005). Charcot–Marie–Tooth neuropathy is also observed in patients with mutations in mitofusin 2 (Mfn2) and the poorly characterized ganglioside-induced differentiation-associated protein 1 (GDAP1), which both promote mitochondrial dynamics (Pareyson et al., 2013). Mutations in Drp1 also lead to severe neurological disorders, including encephalopathy and refractory epilepsy (Vanstone et al., 2016; Waterham et al., 2007; Yoon et al., 2016). Recently, a patient with mutations in Dnm2 who presented with centronuclear myopathy and cardiomyopathy was found to also have multiple mtDNA deletions, consistent with the involvement of Dnm2 in mitochondrial function (Gal et al., 2015).

A reappraisal of mitochondrial fission is that Drp1 and its adaptors are part of a ‘mitochondrial constrictase machinery’, whereas Dnm2 is a scission mediator (Fig. 2). This raises the question of how then is Dnm2 recruited to the constricted mitochondrial membrane? Endophilins have been described to cooperate with dynamins in the endocytic pathway (Farsad et al., 2001; Ringstad et al., 1997; Rostovtseva et al., 2009; Sundborger et al., 2011) and to be able to co-oligomerize with dynamin on lipid tubules (Farsad et al., 2001; Sundborger et al., 2011). Endophilin B1 (also called Bif-1 and SH3GLB1) can be found on the mitochondrial outer membrane and has been shown to be involved in the maintenance of mitochondrial morphology (Karbowski et al., 2004). Following depletion of endophilin B1, the dynamics of the outer mitochondrial membrane is impaired, with the dissociation of the mitochondrial outer membrane from the matrix and the formation of tubules emanating from mitochondria (Karbowski et al., 2004). Given the dual role of Dnm2 in both endocytosis and mitochondrial fission and the importance of endophilin proteins in both processes, it is plausible that endophilin B1 could act as a Dnm2 adaptor for mitochondrial fission.

Role of the ER machinery in driving mitochondrial constriction

Mitochondrial tubules have varying diameters, and in human cells, they are often ∼300 nm. Therefore, the polymeric rings assembled by neither Dnm2 nor Drp1 are sufficiently large in diameter to be able to wrap around non-constricted mitochondria. To achieve mitochondrial fission, a ‘pre-Drp1 constriction step’ is necessary, which reduces the diameter to ∼150 nm (Friedman et al., 2011). In this process, multiple proteins and cellular structures coordinate pre-constriction of the mitochondrial outer membrane (Fig. 2). By performing electron microscopy (EM) tomography and live-cell imaging, Voeltz and co-workers have shown that the ER wraps around mitochondria to facilitate constriction of the underlying tubule (Friedman et al., 2011). These pre-constrictions mark potential sites for mitochondrial fission. This step is independent of and upstream from the assembly of Drp1 and mitochondrial fission adaptors, as pre-constriction can be observed in cell lines in which Drp1, Mff, MiD49 and MiD51 have been knocked down or knocked out (Friedman et al., 2011; Osellame et al., 2016). Further investigation by other groups has led to the identification of an ER-associated machinery that engages in mitochondrial constriction through the concerted action of actin and myosin (Hatch et al., 2014; Phillips and Voeltz, 2016). The ER-associated form of inverted formin 2 (INF2) has been shown to be important for mitochondrial fission (Korobova et al., 2013) in that it serves as a nucleation centre for actin polymerization (Li et al., 2015). In addition, a particular isoform of Spire1, Spire1C, acts a mitochondria-localized actin nucleator and binds to INF2 to promote actin polymerization (Manor et al., 2015). It was proposed that Spire1C interacts with INF2 to give rise to polymerizing actin fibres, which elongate to exert pressure on the mitochondrial outer membrane, resulting in mitochondrial constriction (Manor et al., 2015). However, this raises the question of where the energy for pre-constriction comes from. Higgs and colleagues have also demonstrated the additional involvement of myosin IIA and IIB in constriction of the mitochondrial tubule and recruitment of Drp1 (Korobova et al., 2014). Therefore, contraction of myosin II fibres could pull the tethered membranes together, thus delivering the forces that are necessary for mitochondrial pre-constriction.

Journal of Cell Science (2017) 130, 2953-2960 doi:10.1242/jcs.199562

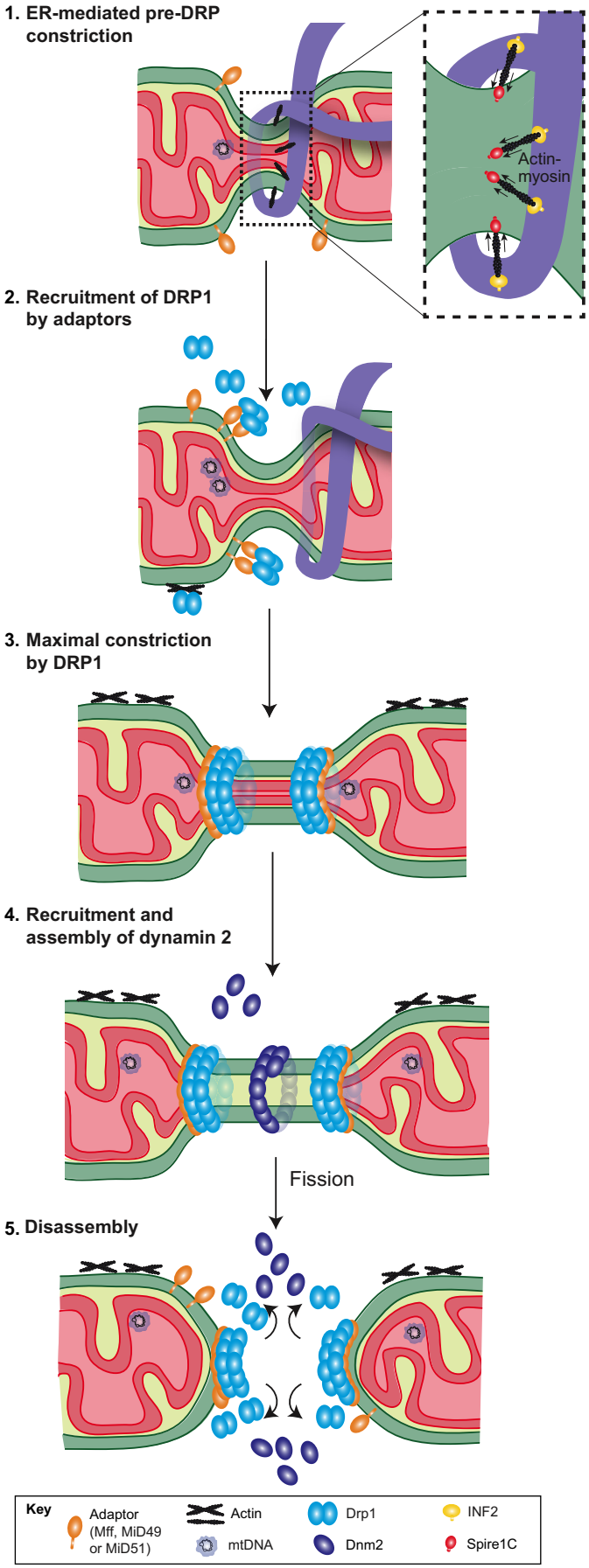

Fig. 2. Model for mitochondrial fission in animal cells. Step 1, the 'pre-Drp1' constriction of mitochondria by the ER constriction machinery takes place, with potential cross-talk between replicating mitochondrial nucleoids. The enlarged view depicts ER-localized INF2 initiating the nucleation of actin filaments, which may associate with mitochondria through Spire1C. Growing actin filaments, together with myosin II, exert pressure on the mitochondrial outer membrane. Step 2, the future fission site is marked for further constriction. This involves binding of the mitochondrial fission adaptor Mff at the neck, which facilitates the recruitment of Drp1. MiD49 and/or MiD51 may also be recruited at this time. Localized changes in lipid composition (e.g. increased cardiolipin) may also facilitate Drp1 assembly. Following adaptor assembly, Drp1 dimers are recruited to the fission site. Different oligomeric forms of Drp1 may be recruited to the mitochondrial surface, including some by actin filaments. Adaptors such as MiD49 and MiD51 may enhance the assembly of the Drp1 contractile ring at the membrane neck. Step 3, GTP hydrolysis fuels the conformational changes, thereby mediating Drp1 constriction, which may enable inner membrane scission to take place. Step 4, following this, Dnm2 is recruited to the constricted mitochondrial neck. Here, additional Dnm2 adaptor proteins might be involved. Step 5, upon GTP hydrolysis, further constriction occurs to complete fission, before the fission machinery is disassembled.

Other roles for actin in mitochondrial fission

While the recent data discussed above implicate actomyosin-mediated forces in ER-derived mitochondrial constriction, actin appears to play important roles in other aspects of mitochondrial fission. For example, it has been previously demonstrated that disruption of F-actin reduces global mitochondrial fission by impairing the recruitment of Drp1 to mitochondria (De Vos et al., 2005). In *Drosophila* egg extracts, actin can be pulled down with Drp1 (DuBoff et al., 2012), and actin can directly stimulate the GTP hydrolysis rate of Drp1 (Hatch et al., 2016; Ji et al., 2015). While most studies have focused on the link between actin and Drp1, it is also well established that Dnm2 influences actin dynamics at the plasma membrane (Gu et al., 2010; Schafer, 2004). Future investigations into the Dnm2 function in mitochondrial fission may also uncover important links with actin.

Besides their direct engagement at the fission site, actin filaments have also been found to transiently cover mitochondria and influence fission events. F-actin has been found to accumulate on mitochondria in cells lacking Drp1 and to transiently accumulate on dividing mitochondria (Li et al., 2015). More recently, it was reported that F-actin can transiently 'swarm' through different areas of the cell, and, when it covers mitochondrial subpopulations, Drp1-dependent mitochondrial fission ensues (Moore et al., 2016). Actin also appears to form a cage around newly divided mitochondria, which may provide a recovery step following fission, for example, by preventing premature fusion, trafficking or mitophagy of the new organelle (Moore et al., 2016). The multiple and varied roles proposed for actin and other cytoskeletal proteins in mitochondrial fission will surely be clarified through additional mechanistic studies.

Additional layers of complexity – lipids and mitochondrial fission

The connection between mitochondrial fission and the lipid environment was first uncovered by *in vitro* studies showing that liposomes with a lipid composition resembling the outer mitochondrial membrane could stimulate Dnm1 self-assembly (Lackner et al., 2009). Cardiolipin is a negatively charged phospholipid, exclusively found in mitochondria, where it comprises 14 to 23% of lipids in the inner mitochondrial membrane and a small fraction of lipids in the outer mitochondrial membrane (Daum, 1985). *In vitro* experiments showed that Drp1 can directly interact with cardiolipin-enriched

membrane vesicles via its B-insert, resulting in the stimulation of its GTPase activity (Bustillo-Zabalbeitia et al., 2014; Macdonald et al., 2014). Since knockout of the adaptors Mff, MiD49 and MiD51 lead to decreased Drp1 levels at mitochondria and fission defects, cardiolipin does not appear to be sufficient for the recruitment of Drp1 onto mitochondria, but rather is likely to have regulatory functions. Indeed, recent research showed that the stimulatory effects of Mff on the different Drp1 isoforms is influenced by cardiolipin levels (Macdonald et al., 2015). Finally, it was recently shown that the conversion of cardiolipin into phosphatidic acid by the mitochondrial phospholipase D (mitoPLD; also known as PLD6) has inhibitory effects on Drp1-mediated fission (Adachi et al., 2016). Increased levels of saturated lipids, such as phosphatidic acid, suppress the GTPase activity of Drp1 at mitochondria and may lead to reduced fission (Adachi et al., 2016). Interestingly, increased phosphatidic acid has also been shown to stimulate mitochondrial fusion (Choi et al., 2006). These results point to a common mechanism by which the opposing processes of fission and fusion can be regulated.

Fission of a double-membraned organelle – the involvement of other players?

At the point where dynamin-mediated constriction induces contacts between two phospholipid bilayers, the inner phospholipid leaflet of the membrane becomes destabilized, while the outer leaflet remains intact. At this stage, it has been proposed that a metastable hemi-fission intermediate forms where opposing inner leaflets of the membrane fuse (Mattila et al., 2015). The fusion of the leaflets before complete division will prevent any leakage of contents from the lumen. Mitochondria bring additional complexity to the general process of membrane fission, as the inner membrane needs to divide before the outer membrane. As constriction of mitochondria occurs from the cytosolic side, the outer membrane will push non-divided inner membranes closer together (Fig. 2). The distance necessary for spontaneous membrane fission between juxtaposed leaflets of a lipid bilayer is ∼1 nm (Bashkirov et al., 2008). However, membranes have a diameter of ∼4 nm, suggesting that the mitochondrion would need to constrict to an external diameter of ∼20 nm, before the inner membrane could divide. During such a constriction, the outer membrane would also make contacts with the inner membrane, which may be conducive to undesirable hemi-fission occurring between the two membranes. Therefore, mechanisms must be in place to prevent the joining of the inner and outer membranes during the process, as otherwise matrix contents would spill out into the cytosol. The very nature of mitochondrial organization shows that mitochondria have closely juxtaposed inner and outer membranes, yet these membranes do not mix, indicating that intrinsic forces somehow prevent this. The lipid asymmetry between the outer and inner membranes, such as the higher cardiolipin content in the inner membrane, is likely to be an important factor. The possibility also exists that additional proteins are involved in coordinating inner membrane fission. Opa1 is a dynamin-related GTPase of the inner membrane that has been implicated as having roles in both mitochondrial fission and fusion (Pernas and Scorrano, 2016). Recently, it was shown that the short processed isoform of inner-membrane associated Opa1 (S-Opa1) promotes mitochondrial fission (Anand et al., 2014). Interestingly, S-Opa1 was found at mitochondrial constriction sites along with MiD49, Drp1 and the ER, thus suggesting that there is a level of communication between the inner and outer membrane division machineries (Anand et al., 2014). A possible link may be via MiD51, as it was shown that the transmembrane anchor of MiD51 is required for correct positioning of the fission apparatus with the Opa1-mediated cristae remodelling machinery during apoptosis (Otera et al., 2016).

A further element of fission regulation extends into the mitochondrial matrix. It has recently been found that new mtDNA nucleoids generated by the mitochondrial DNA polymerase subunit γ2 (POLG2) are preferentially associated at sites where mitochondrial division takes place (Lewis et al., 2016). Moreover, these newly synthesized mtDNA nucleoids were found at mitochondrial constriction sites that also involve the ER (Lewis et al., 2016). This suggests the presence of a signalling network of proteins involved in communicating the replication of mtDNA nucleoids in the matrix to the mitochondrial fission machinery. The key proteins involved in such signalling await identification.

Regulation of the fission machinery

It is well established that posttranslational modifications and protein degradation play an important role in the regulation of mitochondrial fission. Most notably, Drp1 has the potential to undergo significant modification. Several SUMOylation, S-nitrosylation and phosphorylation sites have been identified in Drp1, with these predominantly located in the B-insert (Chang and Blackstone, 2010; Cho et al., 2009; Figueroa-Romero et al., 2009; Fröhlich et al., 2013; Otera et al., 2013; Prudent et al., 2015). Phosphorylation of Drp1 has been investigated extensively and can have either inhibitory or enhancing effects, depending on the specific residue being modified. Phosphorylation of S616 enhances mitochondrial fission, whereas phosphorylation of S637 by protein kinase A (PKA) inhibits the GTPase hydrolysis activity of Drp1 *in vitro*, leading to elongation of mitochondria (Chang and Blackstone, 2007; Cribbs and Strack, 2007). Furthermore, dephosphorylation of S637 by the Ca^{2+}-dependent phosphatase calcineurin activates Drp1 recruitment to the outer mitochondrial surface and fission (Cereghetti et al., 2008; Cribbs and Strack, 2007; Mishra and Chan, 2016). Thus, metabolic stimuli signalled through Ca^{2+} fluctuations are translated into alterations of mitochondrial morphology.

Given its extensive modifications, it might be expected that fission would be solely under the regulatory control of Drp1. However, there is evidence that the regulation of fission is also mediated by the Drp1 adaptors. Recently, Mff has been found to serve as a linker between energy stress and mitochondrial morphology. A screen for substrates of the energy-sensing AMP protein kinase (AMPK) revealed the phosphorylation of Mff, leading to its subsequent activation and enhanced mitochondrial fission (Toyama et al., 2016). In separate structural studies, MiD51 was found to have an inactive nucleotidyltransferase fold, yet still has the capacity to bind both to GDP and ADP (Losón et al., 2014; Richter et al., 2014). The physiological relevance of this is unclear, with one study showing that Drp1 recruitment to mitochondria was blocked in the absence of ADP (Losón et al., 2014), whereas another study found that expression of MiD51 mutants lacking nucleotide binding could still recruit Drp1 to mitochondria to promote fission (Richter et al., 2014). The structure of MiD49 indicates that it lacks the ability for nucleotide binding altogether (Losón et al., 2015). However, MiD49 levels are influenced by the E3 ubiquitin ligase MARCH5, which in turn is regulated by Mff and Drp1 (Cherok et al., 2017; Xu et al., 2016). The involvement of multiple signalling platforms for fission may be explained by the diverse roles for mitochondrial fission in cellular biology and variations in tissue-specific expression and activity of fission members (Macdonald et al., 2015).

Conclusion

In the past decade, the number of components involved in mitochondrial fission has rapidly expanded. This includes new adaptor proteins, the involvement of an ER-associated machinery, F-actin associations and, importantly, a new mediator of membrane scission, Dnm2. Currently, the mechanisms by which Dnm2 is recruited to fission sites is unknown. Because Dnm2 is also crucial for endocytic events at the plasma membrane, this raises the question of whether it is recruited from the plasma membrane by the cytoskeleton, or whether there are distinct Dnm2 pools for functions at either the periphery or in the cell lumen. The role of Dnm2 in mitochondrial fission also further begs the question as to whether Dnm2 also performs peroxisomal division together with Drp1. In humans, three classical dynamin proteins exist: Dnm2 is ubiquitously expressed, whereas dynamin-1 and dynamin-3 are mainly expressed in neurons (Ferguson et al., 2007). It will therefore be interesting to determine whether dynamin-1 and dynamin-3 also exert functions in mitochondrial fission in a tissue-specific manner. A potential role for the yeast dynamin Vps1 in mitochondrial fission has yet to be established (Smaczynska-de et al., 2010).

Exciting new findings show that mitochondrial fission has a role in the inheritance of mtDNA (Lewis et al., 2016). The mechanisms underlying the spatiotemporal synchronization of mtDNA replication in the matrix with the pre-Drp1 constriction apparatus involving the ER is currently unknown but is likely to be a major focus of future research endeavours.

Finally, additional studies aimed at elucidating the role of actin in fission are required. As mentioned above, actin has been found to be involved in multiple events of the fission cycle including in ER constriction and Drp1 recruitment, as well as after scission. Given the well-known associations between dynamins and actin, such an association may also provide torsional stresses to assist with the actual Dnm2-mediated scission step. Further investigations using biochemical and new imaging approaches will give new insights, helping us to understand the mechanism and regulation of dynamics of the cellular powerhouse.

Competing interests

The authors declare no competing or financial interests.

Funding

Our work in this area was supported with funding from the Australian Research Council (grant number DP160102176) and the National Health and Medical Research Council.

References

Adachi, Y., Itoh, K., Yamada, T., Cerveny, K. L., Suzuki, T. L., Macdonald, P., Frohman, M. A., Ramachandran, R., Iijima, M. and Sesaki, H. (2016). Coincident phosphatidic acid interaction restrains Drp1 in mitochondrial division. *Mol. Cell* **63**, 1034-1043.

Anand, R., Wai, T., Baker, M. J., Kladt, N., Schauss, A. C., Rugarli, E. and Langer, T. (2014). The i-AAA protease YME1L and OMA1 cleave OPA1 to balance mitochondrial fusion and fission. *J. Cell Biol.* **204**, 919-929.

Antonny, B., Burd, C., De Camilli, P., Chen, E., Daumke, O., Faelber, K., Ford, M., Frolov, V. A., Frost, A., Hinshaw, J. E. et al. (2016). Membrane fission by dynamin: what we know and what we need to know. *EMBO J.* **35**, 2270-2284.

Bashkirov, P. V., Akimov, S. A., Evseev, A. I., Schmid, S. L., Zimmerberg, J. and Frolov, V. A. (2008). GTPase cycle of dynamin is coupled to membrane squeeze and release, leading to spontaneous fission. *Cell* **135**, 1276-1286.

Bitoun, M., Maugenre, S., Jeannet, P.-Y., Lacène, E., Ferrer, X., Laforêt, P., Martin, J.-J., Laporte, J., Lochmüller, H., Beggs, A. H. et al. (2005). Mutations in dynamin 2 cause dominant centronuclear myopathy. *Nat. Genet.* **37**, 1207-1209.

Buck, M. D., O'Sullivan, D., Klein Geltink, R. I., Curtis, J. D., Chang, C.-H., Sanin, D. E., Qiu, J., Kretz, O., Braas, D., van der Windt, G. J. W. et al. (2016). Mitochondrial dynamics controls T cell fate through metabolic programming. *Cell* **166**, 63-76.

Bustillo-Zabalbeitia, I., Montessuit, S., Raemy, E., Basañez, G., Terrones, O. and Martinou, J.-C. (2014). Specific interaction with cardiolipin triggers functional activation of Dynamin-Related Protein 1. *PLoS ONE* **9**, e102738.

Cereghetti, G. M., Stangherlin, A., Martins de Brito, O., Chang, C. R., Blackstone, C., Bernardi, P. and Scorrano, L. (2008). Dephosphorylation by calcineurin regulates translocation of Drp1 to mitochondria. *Proc. Natl. Acad. Sci. USA* **105**, 15803-15808.

Chang, C.-R. and Blackstone, C. (2007). Cyclic AMP-dependent protein kinase phosphorylation of Drp1 regulates its GTPase activity and mitochondrial morphology. *J. Biol. Chem.* **282**, 21583-21587.

Chang, C.-R. and Blackstone, C. (2010). Dynamic regulation of mitochondrial fission through modification of the dynamin-related protein Drp1. *Ann. N. Y. Acad. Sci.* **1201**, 34-39.

Chen, H., Ren, S., Clish, C., Jain, M., Mootha, V., McCaffery, J. M. and Chan, D. C. (2015). Titration of mitochondrial fusion rescues Mff-deficient cardiomyopathy. *J. Cell Biol.* **211**, 795-805.

Cherok, E., Xu, S., Li, S., Das, S., Meltzer, W. A., Zalzman, M., Wang, C. and Karbowski, M. (2017). Novel regulatory roles of Mff and Drp1 in E3 ubiquitin ligase MARCH5-dependent degradation of MiD49 and Mcl1 and control of mitochondrial dynamics. *Mol. Biol. Cell* **28**, 396-410.

Cho, D.-H., Nakamura, T., Fang, J., Cieplak, P., Godzik, A., Gu, Z. and Lipton, S. A. (2009). S-nitrosylation of Drp1 mediates beta-amyloid-related mitochondrial fission and neuronal injury. *Science* **324**, 102-105.

Choi, S.-Y., Huang, P., Jenkins, G. M., Chan, D. C., Schiller, J. and Frohman, M. A. (2006). A common lipid links Mfn-mediated mitochondrial fusion and SNARE-regulated exocytosis. *Nat. Cell Biol.* **8**, 1255-1262.

Cribbs, J. T. and Strack, S. (2007). Reversible phosphorylation of Drp1 by cyclic AMP-dependent protein kinase and calcineurin regulates mitochondrial fission and cell death. *EMBO Rep.* **8**, 939-944.

Daum, G. (1985). Lipids of mitochondria. *Biochim. Biophys. Acta* **822**, 1-42.

De Vos, K. J., Allan, V. J., Grierson, A. J. and Sheetz, M. P. (2005). Mitochondrial function and actin regulate dynamin-related protein 1-dependent mitochondrial fission. *Curr. Biol.* **15**, 678-683.

DuBoff, B., Götz, J. and Feany, M. B. (2012). Tau promotes neurodegeneration via DRP1 mislocalization in vivo. *Neuron* **75**, 618-632.

Durieux, A.-C., Prudhon, B., Guicheney, P. and Bitoun, M. (2010). Dynamin 2 and human diseases. *J. Mol. Med. (Berl.)* **88**, 339-350.

Farsad, K., Ringstad, N., Takei, K., Floyd, S. R., Rose, K. and De Camilli, P. (2001). Generation of high curvature membranes mediated by direct endophilin bilayer interactions. *J. Cell Biol.* **155**, 193-200.

Ferguson, S. M., Brasnjo, G., Hayashi, M., Wolfel, M., Collesi, C., Giovedi, S., Raimondi, A., Gong, L.-W., Ariel, P., Paradise, S. et al. (2007). A selective activity-dependent requirement for dynamin 1 in synaptic vesicle endocytosis. *Science* **316**, 570-574.

Figueroa-Romero, C., Iniguez-Lluhi, J. A., Stadler, J., Chang, C.-R., Arnoult, D., Keller, P. J., Hong, Y., Blackstone, C. and Feldman, E. L. (2009). SUMOylation of the mitochondrial fission protein Drp1 occurs at multiple nonconsensus sites within the B domain and is linked to its activity cycle. *FASEB J.* **23**, 3917-3927.

Ford, M. G. J., Jenni, S. and Nunnari, J. (2011). The crystal structure of dynamin. *Nature* **477**, 561-566.

Friedman, J. R. and Nunnari, J. (2014). Mitochondrial form and function. *Nature* **505**, 335-343.

Friedman, J. R., Lackner, L. L., West, M., DiBenedetto, J. R., Nunnari, J. and Voeltz, G. K. (2011). ER tubules mark sites of mitochondrial division. *Science* **334**, 358-362.

Fröhlich, C., Grabiger, S., Schwefel, D., Faelber, K., Rosenbaum, E., Mears, J., Rocks, O. and Daumke, O. (2013). Structural insights into oligomerization and mitochondrial remodelling of dynamin 1-like protein. *EMBO J.* **32**, 1280-1292.

Gal, A., Inczedy-Farkas, G., Pal, E., Remenyi, V., Bereznai, B., Geller, L., Szelid, Z., Merkely, B. and Molnar, M. J. (2015). The coexistence of dynamin 2 mutation and multiple mitochondrial DNA (mtDNA) deletions in the background of severe cardiomyopathy and centronuclear myopathy. *Clin. Neuropathol.* **34**, 89-95.

Gandre-Babbe, S. and van der Bliek, A. M. (2008). The novel tail-anchored membrane protein Mff controls mitochondrial and peroxisomal fission in mammalian cells. *Mol. Biol. Cell* **19**, 2402-2412.

Gomes, L. C., Di Benedetto, G. and Scorrano, L. (2011). During autophagy mitochondria elongate, are spared from degradation and sustain cell viability. *Nat. Cell Biol.* **13**, 589-598.

Gu, C., Yaddanapudi, S., Weins, A., Osborn, T., Reiser, J., Pollak, M., Hartwig, J. and Sever, S. (2010). Direct dynamin-actin interactions regulate the actin cytoskeleton. *EMBO J.* **29**, 3593-3606.

Hatch, A. L., Gurel, P. S. and Higgs, H. N. (2014). Novel roles for actin in mitochondrial fission. *J. Cell Sci.* **127**, 4549-4560.

Hatch, A. L., Ji, W.-K., Merrill, R. A., Strack, S. and Higgs, H. N. (2016). Actin filaments as dynamic reservoirs for Drp1 recruitment. *Mol. Biol. Cell* **27**, 3109-3121.

Ingerman, E., Perkins, E. M., Marino, M., Mears, J. A., McCaffery, J. M., Hinshaw, J. E. and Nunnari, J. (2005). Dnm1 forms spirals that are structurally tailored to fit mitochondria. *J. Cell Biol.* **170**, 1021-1027.

Ishihara, N., Nomura, M., Jofuku, A., Kato, H., Suzuki, S. O., Masuda, K., Otera, H., Nakanishi, Y., Nonaka, I., Goto, Y. et al. (2009). Mitochondrial fission factor Drp1 is essential for embryonic development and synapse formation in mice. *Nat. Cell Biol.* **11**, 958-966.

Ji, W. K., Hatch, A. L., Merrill, R. A., Strack, S. and Higgs, H. N. (2015). Actin filaments target the oligomeric maturation of the dynamin GTPase Drp1 to mitochondrial fission sites. *Elife* **4**, e11553.

Kageyama, Y., Zhang, Z., Roda, R., Fukaya, M., Wakabayashi, J., Wakabayashi, N., Kensler, T. W., Reddy, P. H., Iijima, M. and Sesaki, H. (2012). Mitochondrial division ensures the survival of postmitotic neurons by suppressing oxidative damage. *J. Cell Biol.* **197**, 535-551.

Karbowski, M., Jeong, S.-Y. and Youle, R. J. (2004). Endophilin B1 is required for the maintenance of mitochondrial morphology. *J. Cell Biol.* **166**, 1027-1039.

Koch, A., Thiemann, M., Grabenbauer, M., Yoon, Y., McNiven, M. A. and Schrader, M. (2003). Dynamin-like protein 1 is involved in peroxisomal fission. *J. Biol. Chem.* **278**, 8597-8605.

Koirala, S., Guo, Q., Kalia, R., Bui, H. T., Eckert, D. M., Frost, A. and Shaw, J. M. (2013). Interchangeable adaptors regulate mitochondrial dynamin assembly for membrane scission. *Proc. Natl. Acad. Sci. USA* **110**, E1342-E1351.

Korobova, F., Ramabhadran, V. and Higgs, H. N. (2013). An actin-dependent step in mitochondrial fission mediated by the ER-associated formin INF2. *Science* **339**, 464-467.

Korobova, F., Gauvin, T. J. and Higgs, H. N. (2014). A role for myosin II in mammalian mitochondrial fission. *Curr. Biol.* **24**, 409-414.

Lackner, L. L. (2014). Shaping the dynamic mitochondrial network. *BMC Biol.* **12**, 35.

Lackner, L. L., Horner, J. S. and Nunnari, J. (2009). Mechanistic analysis of a dynamin effector. *Science* **325**, 874-877.

Lee, J. E., Westrate, L. M., Wu, H., Page, C. and Voeltz, G. K. (2016). Multiple dynamin family members collaborate to drive mitochondrial division. *Nature* **540**, 139-143.

Legesse-Miller, A., Massol, R. H. and Kirchhausen, T. (2003). Constriction and Dnm1p recruitment are distinct processes in mitochondrial fission. *Mol. Biol. Cell* **14**, 1953-1963.

Lewis, S. C., Uchiyama, L. F. and Nunnari, J. (2016). ER-mitochondria contacts couple mtDNA synthesis with mitochondrial division in human cells. *Science* **353**, aaf5549.

Li, S., Xu, S., Roelofs, B. A., Boyman, L., Lederer, W. J., Sesaki, H. and Karbowski, M. (2015). Transient assembly of F-actin on the outer mitochondrial membrane contributes to mitochondrial fission. *J. Cell Biol.* **208**, 109-123.

Losón, O. C., Song, Z., Chen, H. and Chan, D. C. (2013). Fis1, Mff, MiD49 and MiD51 mediate Drp1 recruitment in mitochondrial fission. *Mol. Biol. Cell* **24**, 659-667.

Losón, O. C., Liu, R., Rome, M. E., Meng, S., Kaiser, J. T., Shan, S.-O. and Chan, D. C. (2014). The mitochondrial fission receptor MiD51 requires ADP as a cofactor. *Structure* **22**, 367-377.

Losón, O. C., Meng, S., Ngo, H., Liu, R., Kaiser, J. T. and Chan, D. C. (2015). Crystal structure and functional analysis of MiD49, a receptor for the mitochondrial fission protein Drp1. *Protein Sci.* **24**, 386-394.

Macdonald, P. J., Stepanyants, N., Mehrotra, N., Mears, J. A., Qi, X., Sesaki, H. and Ramachandran, R. (2014). A dimeric equilibrium intermediate nucleates Drp1 reassembly on mitochondrial membranes for fission. *Mol. Biol. Cell* **25**, 1905-1915.

Macdonald, P. J., Francy, C. A., Stepanyants, N., Lehman, L., Baglio, A., Mears, J. A., Qi, X. and Ramachandran, R. (2015). Distinct splice variants of dynamin-related protein 1 differentially utilize mitochondrial fission factor as an effector of cooperative GTPase activity. *J. Biol. Chem.* **291**, 493-507.

Manor, U., Bartholomew, S., Golani, G., Christenson, E., Kozlov, M., Higgs, H., Spudich, J. and Lippincott-Schwartz, J. (2015). A mitochondria-anchored isoform of the actin-nucleating spire protein regulates mitochondrial division. *Elife* **4**, e08828.

Martinou, J.-C. and Youle, R. J. (2011). Mitochondria in apoptosis: Bcl-2 family members and mitochondrial dynamics. *Dev. Cell* **21**, 92-101.

Mattila, J. P., Shnyrova, A. V., Sundborger, A. C., Hortelano, E. R., Fuhrmans, M., Neumann, S., Muller, M., Hinshaw, J. E., Schmid, S. L. and Frolov, V. A. (2015). A hemi-fission intermediate links two mechanistically distinct stages of membrane fission. *Nature* **524**, 109-113.

Mears, J. A., Lackner, L. L., Fang, S., Ingerman, E., Nunnari, J. and Hinshaw, J. E. (2011). Conformational changes in Dnm1 support a contractile mechanism for mitochondrial fission. *Nat. Struct. Mol. Biol.* **18**, 20-26.

Merrifield, C. J., Perrais, D. and Zenisek, D. (2005). Coupling between clathrin-coated-pit invagination, cortactin recruitment, and membrane scission observed in live cells. *Cell* **121**, 593-606.

Mishra, P. and Chan, D. C. (2016). Metabolic regulation of mitochondrial dynamics. *J. Cell Biol.* **212**, 379-387.

Moore, A. S., Wong, Y. C., Simpson, C. L. and Holzbaur, E. L. (2016). Dynamic actin cycling through mitochondrial subpopulations locally regulates the fission-fusion balance within mitochondrial networks. *Nat. Commun.* **7**, 12886.

Morlot, S., Galli, V., Klein, M., Chiaruttini, N., Manzi, J., Humbert, F., Dinis, L., Lenz, M., Cappello, G. and Roux, A. (2012). Membrane shape at the edge of the dynamin helix sets location and duration of the fission reaction. *Cell* **151**, 619-629.

Naylor, K., Ingerman, E., Okreglak, V., Marino, M., Hinshaw, J. E. and Nunnari, J. (2006). Mdv1 interacts with assembled dnm1 to promote mitochondrial division. *J. Biol. Chem.* **281**, 2177-2183.

Osellame, L. D., Singh, A. P., Stroud, D. A., Palmer, C. S., Stojanovski, D., Ramachandran, R. and Ryan, M. T. (2016). Cooperative and independent roles of the Drp1 adaptors Mff, MiD49 and MiD51 in mitochondrial fission. *J. Cell Sci.* **129**, 2170-2181.

Otera, H., Wang, C., Cleland, M. M., Setoguchi, K., Yokota, S., Youle, R. J. and Mihara, K. (2010). Mff is an essential factor for mitochondrial recruitment of Drp1 during mitochondrial fission in mammalian cells. *J. Cell Biol.* **191**, 1141-1158.

Otera, H., Ishihara, N. and Mihara, K. (2013). New insights into the function and regulation of mitochondrial fission. *Biochim. Biophys. Acta Mol. Cell Res.* **1833**, 1256-1268.

Otera, H., Miyata, N., Kuge, O. and Mihara, K. (2016). Drp1-dependent mitochondrial fission via MiD49/51 is essential for apoptotic cristae remodeling. *J. Cell Biol.* **212**, 531-544.

Palmer, C. S., Osellame, L. D., Laine, D., Koutsopoulos, O. S., Frazier, A. E. and Ryan, M. T. (2011). MiD49 and MiD51, new components of the mitochondrial fission machinery. *EMBO Rep.* **12**, 565-573.

Palmer, C. S., Elgass, K. D., Parton, R. G., Osellame, L. D., Stojanovski, D. and Ryan, M. T. (2013). MiD49 and MiD51 can act independently of Mff and Fis1 in Drp1 recruitment and are specific for mitochondrial fission. *J. Biol. Chem.* **288**, 27584-27593.

Pareyson, D., Piscosquito, G., Moroni, I., Salsano, E. and Zeviani, M. (2013). Peripheral neuropathy in mitochondrial disorders. *Lancet Neurol.* **12**, 1011-1024.

Pernas, L. and Scorrano, L. (2016). Mito-morphosis: mitochondrial fusion, fission, and cristae remodeling as key mediators of cellular function. *Annu. Rev. Physiol.* **78**, 505-531.

Phillips, M. J. and Voeltz, G. K. (2016). Structure and function of ER membrane contact sites with other organelles. *Nat. Rev. Mol. Cell Biol.* **17**, 69-82.

Plecita-Hlavata, L., Lessard, M., Santorova, J., Bewersdorf, J. and Jezek, P. (2008). Mitochondrial oxidative phosphorylation and energetic status are reflected by morphology of mitochondrial network in INS-1E and HEP-G2 cells viewed by 4Pi microscopy. *Biochim. Biophys. Acta* **1777**, 834-846.

Prudent, J. and McBride, H. M. (2017). The mitochondria-endoplasmic reticulum contact sites: a signalling platform for cell death. *Curr. Opin. Cell Biol.* **47**, 52-63.

Prudent, J., Zunino, R., Sugiura, A., Mattie, S., Shore, G. C. and McBride, H. M. (2015). MAPL SUMOylation of Drp1 stabilizes an ER/mitochondrial platform required for cell death. *Mol. Cell.* **59**, 941-955.

Rambold, A. S., Kostelecky, B., Elia, N. and Lippincott-Schwartz, J. (2011). Tubular network formation protects mitochondria from autophagosomal degradation during nutrient starvation. *Proc. Natl. Acad. Sci. USA* **108**, 10190-10195.

Richter, V., Palmer, C. S., Osellame, L. D., Singh, A. P., Elgass, K., Stroud, D. A., Sesaki, H., Kvansakul, M. and Ryan, M. T. (2014). Structural and functional analysis of MiD51, a dynamin receptor required for mitochondrial fission. *J. Cell Biol.* **204**, 477-486.

Richter, V., Singh, A. P., Kvansakul, M., Ryan, M. T. and Osellame, L. D. (2015). Splitting up the powerhouse: structural insights into the mechanism of mitochondrial fission. *Cell. Mol. Life Sci.* **72**, 3695-3707.

Ringstad, N., Nemoto, Y. and De Camilli, P. (1997). The SH3p4/Sh3p8/SH3p13 protein family: binding partners for synaptojanin and dynamin via a Grb2-like Src homology 3 domain. *Proc. Natl. Acad. Sci. USA* **94**, 8569-8574.

Rostovtseva, T. K., Boukari, H., Antignani, A., Shiu, B., Banerjee, S., Neutzner, A. and Youle, R. J. (2009). Bax activates endophilin B1 oligomerization and lipid membrane vesiculation. *J. Biol. Chem.* **284**, 34390-34399.

Roy, M., Reddy, P. H., Iijima, M. and Sesaki, H. (2015). Mitochondrial division and fusion in metabolism. *Curr. Opin. Cell Biol.* **33**, 111-118.

Schafer, D. A. (2004). Regulating actin dynamics at membranes: a focus on dynamin. *Traffic* **5**, 463-469.

Smaczynska-de, R., Il, Allwood, E. G., Aghamohammadzadeh, S., Hettema, E. H., Goldberg, M. W. and Ayscough, K. R. (2010). A role for the dynamin-like protein Vps1 during endocytosis in yeast. *J. Cell Sci.* **123**, 3496-3506.

Smirnova, E., Griparic, L., Shurland, D. L. and van der Bliek, A. M. (2001). Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells. *Mol. Biol. Cell* **12**, 2245-2256.

Sundborger, A., Soderblom, C., Vorontsova, O., Evergren, E., Hinshaw, J. E. and Shupliakov, O. (2011). An endophilin-dynamin complex promotes budding of clathrin-coated vesicles during synaptic vesicle recycling. *J. Cell Sci.* **124**, 133-143.

Sundborger, A. C., Fang, S., Heymann, J. A., Ray, P., Chappie, J. S. and Hinshaw, J. E. (2014). A dynamin mutant defines a superconstricted prefission state. *Cell Rep.* **8**, 734-742.

Sweitzer, S. M. and Hinshaw, J. E. (1998). Dynamin undergoes a GTP-dependent conformational change causing vesiculation. *Cell* **93**, 1021-1029.

Toyama, E. Q., Herzig, S., Courchet, J., Lewis, T. L., Jr, Losón, O. C., Hellberg, K., Young, N. P., Chen, H., Polleux, F., Chan, D. C. et al. (2016). Metabolism.

AMP-activated protein kinase mediates mitochondrial fission in response to energy stress. *Science* **351**, 275-281.

Twig, G., Elorza, A., Molina, A. J. A., Mohamed, H., Wikstrom, J. D., Walzer, G., Stiles, L., Haigh, S. E., Katz, S., Las, G. et al. (2008). Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *EMBO J.* **27**, 433-446.

Vanstone, J. R., Smith, A. M., McBride, S., Naas, T., Holcik, M., Antoun, G., Harper, M.-E., Michaud, J., Sell, E., Chakraborty, P. et al. (2016). DNM1L-related mitochondrial fission defect presenting as refractory epilepsy. *Eur. J. Hum. Genet.* **24**, 1084-1088.

Wakabayashi, J., Zhang, Z., Wakabayashi, N., Tamura, Y., Fukaya, M., Kensler, T. W., Iijima, M. and Sesaki, H. (2009). The dynamin-related GTPase Drp1 is required for embryonic and brain development in mice. *J. Cell Biol.* **186**, 805-816.

Waterham, H. R., Koster, J., van Roermund, C. W. T., Mooyer, P. A. W., Wanders, R. J. A. and Leonard, J. V. (2007). A lethal defect of mitochondrial and peroxisomal fission. *N. Engl. J. Med.* **356**, 1736-1741.

Xu, S., Cherok, E., Das, S., Li, S., Roelofs, B. A., Ge, S. X., Polster, B. M., Boyman, L., Lederer, W. J., Wang, C. et al. (2016). Mitochondrial E3 ubiquitin ligase MARCH5 controls mitochondrial fission and cell sensitivity to stress-induced apoptosis through regulation of MiD49 protein. *Mol. Biol. Cell* **27**, 349-359.

Yoon, Y., Pitts, K. R. and McNiven, M. A. (2001). Mammalian dynamin-like protein DLP1 tubulates membranes. *Mol. Biol. Cell* **12**, 2894-2905.

Yoon, G., Malam, Z., Paton, T., Marshall, C. R., Hyatt, E., Ivakine, Z., Scherer, S. W., Lee, K. S., Hawkins, C., Cohn, R. D. et al. (2016). Lethal disorder of mitochondrial fission caused by mutations in DNM1L. *J. Pediatr.* **171**, 313-316.e1-2.

Zhang, P. and Hinshaw, J. E. (2001). Three-dimensional reconstruction of dynamin in the constricted state. *Nat. Cell Biol.* **3**, 922-926.

Züchner, S., Noureddine, M., Kennerson, M., Verhoeven, K., Claeys, K., Jonghe, P. D., Merory, J., Oliveira, S. A., Speer, M. C., Stenger, J. E. et al. (2005). Mutations in the pleckstrin homology domain of dynamin 2 cause dominant intermediate Charcot-Marie-Tooth disease. *Nat. Genet.* **37**, 289-294.