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Mitochondrial dynamics: shaping and remodeling an organelle network

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Abstract

Mitochondria form networks that continually remodel and adapt to carry out their cellular function. The mitochondrial network is remodeled by changes in mitochondrial morphology, number, and distribution within the cell. Mitochondrial dynamics depend directly on fission, fusion, shape transition, and transport or tethering along the cytoskeleton. Over the past several years, many of the mechanisms underlying these processes have been uncovered. It has become clear that each process is precisely and contextually regulated within the cell. Here, we discuss the mechanisms regulating each aspect of mitochondrial dynamics, which together shape the network as a whole.

Keywords

mitochondria; morphology; fission; fusion; cytoskeleton; transport

Introduction

Mitochondria are highly dynamic organelles that morphologically adapt to fit cellular needs. The mitochondrial network changes in response to diverse cellular pathways, such as metabolism, intracellular calcium signaling, apoptosis, mitosis, and mitochondrial DNA replication. Despite the diversity of contexts that alter mitochondrial dynamics, the resultant effects on the mitochondrial network are dependent on four distinct processes. Fission, the division of a single mitochondrion into two mitochondria by cleavage of the Inner Mitochondrial Membrane (IMM) and Outer Mitochondrial Membrane (OMM), and fusion, the joining of the OMM and IMM, are in equilibrium to determine network connectivity.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Network morphology is simultaneously determined by mitochondrial shape transitions independent of fission/fusion and precise positioning along the cytoskeleton (Fig. 1). As our understanding of each process develops, we must also establish a holistic understanding of how mitochondrial dynamics are orchestrated to control network properties. Here, we highlight recent progress that provides new insights into the complexity of each aspect of mitochondrial dynamics.

Dividing mitochondria with Drp1 and actin

The key events of mitochondrial fission are constriction and scission of both the OMM and IMM. Outer membrane constriction is driven by Drp1, a GTPase that dynamically associates with the endoplasmic reticulum (ER) and mitochondria (Fig. 2A)[1,2]. Drp1 is recruited to mitochondria via interactions with receptors in the OMM: mitochondrial fission factor (MFF) and mitochondrial dynamics proteins 49 and 51 (MID49/51)[3–6]. Some Drp1 is transferred to the OMM following MFF-dependent oligomerization on the ER; this transfer likely occurs at mitochondria-ER contact sites, which mark sites of mitochondrial division (Fig. 2B)[2,7]. Drp1-dependent fission at mitochondria-ER contacts is facilitated by actin assembly, as inhibiting actin polymerization reduces fission frequency and Drp1 recruitment to mitochondria[1,2,8,9]. Actin assembly at mitochondria-ER contacts depends on two actin nucleating proteins, the formin INF2 and Spire1C, which reside on the ER and mitochondria, respectively. These proteins interact to promote actin assembly and mitochondrial constriction (Fig. 2B)[8,10]. Actin filaments locally assemble in a wave-like manner around mitochondrial subpopulations to induce fission[11]. Following actin disassembly, these mitochondrial subpopulations undergo fusion to locally remodel the mitochondrial network.

Recent advances in electron microscopy have revealed the three-dimensional ultrastructure of the actin cytoskeleton during mitochondrial constriction. Yang and Svitkina (2019) found dense arrays of filamentous actin with criss-cross orientation at mitochondrial constrictions[12]. Many of these actin filaments extend from the nearby ER. This study also examined the positioning of non-muscle myosin II (NMII), as this motor has been implicated along with actin and INF2 in fission[1,13]. NMII is located near mitochondrial constrictions, primarily along the interstitial actin network (Fig. 2B–C), and is proposed to pull on the interstitial actin network to deform mitochondria upstream of Drp1, consistent with findings that NMII promotes Drp1 recruitment to mitochondria[13].

Once recruited, Drp1 oligomerizes to wrap around the outer membrane (Fig. 2C). Upon GTP hydrolysis, Drp1 changes conformation, dissociating MID49/51 to shrink the oligomeric ring (Fig. 2D)[14]. While the Drp1 ring constricts the OMM, there is debate as to whether Drp1 carries out membrane scission. Initial studies found no evidence that Drp1 could drive membrane scission. Dynamin-2 (Dnm2), another dynamin GTPase, was found at fission sites following Drp1 recruitment; Dnm2 knockdown was also found to inhibit mitochondrial fission[15]. However, several recent studies implicate Drp1 as the protein responsible for membrane scission. Fibroblasts lacking Dnm2 or all three dynamin proteins display normal mitochondrial division, suggesting dynamins 1–3 are dispensable for fission[16,17]; in contrast, Drp1 is required for fission[9,16,17]. Further, purified Drp1 can induce

the fission of membrane tubules up to 250 nm in radius[17]. While these results implicate Drp1 as the protein responsible for scission, more work is required to confirm whether Drp1 drives the final step in fission.

Whereas outer membrane scission depends on Drp1 oligomerization and GTP hydrolysis, the mechanism of inner membrane scission is less clear. Recent studies have shown that the IMM constricts and divides at mitochondria-ER contacts prior to Drp1-dependent OMM fission[18,19]. IMM constriction depends on INF2-mediated actin polymerization and NMII, similar to outer membrane constriction. Actin assembly at mitochondria-ER contacts stimulates calcium release from the ER and subsequent mitochondrial uptake through the mitochondrial calcium uniporter (MCU; Fig. 2B)[18]. Elevated mitochondrial calcium then stimulates IMM constriction in a Drp1-independent manner, but the subsequent mechanism of IMM scission is a black box.

While it is clear that mitochondrial fission is largely coordinated by the ER, several other factors determine sites of fission. Fission relies on the dynamic recruitment of lysosomes and the lysosomal GTPase RAB7. GTP-bound RAB7 is recruited to mitochondria by the mitochondrial fission protein 1 (Fis1), an OMM protein with two tetratricopeptide repeat domains exposed to the cytosol[20]. Once recruited, GTP-bound RAB7 promotes mitochondria-lysosome contact formation[21]. Mitochondria-lysosome contacts restrict mitochondrial motility, regulate inter-mitochondrial tethering, and mark sites of fission[22]. Fission is also modulated by the dynamic recruitment of the trans-Golgi network (TGN). The small GTPase ADP-ribosylation factor 1 (Arf1) and its effector, phosphatidylinositol 4-kinase-III-b [PI(4)KIIIb] are recruited to fission sites on TGN vesicles after Drp1 recruitment[23]. Loss of Arf1 or PI(4)KIIIb produces a hyperfused and branched network, suggesting these proteins affect mitochondrial branching in addition to fission. Intriguingly, TGN vesicles converged with lysosomes and ER at fission sites. Each of these organelles is present at most, but not all mitochondrial fission sites. Further analysis of the temporal and spatial dynamics of these organelles and their effector proteins is necessary to understand how they are coordinated to promote fission.

Promoting fusion or inhibiting fission: a balancing act

Mitochondrial fusion is mediated by the dynamin family GTPases mitofusin 1 (Mfn1), mitofusin 2 (Mfn2), and Opa1. Fusion begins with Mfn1/2-mediated OMM tethering and merging followed by Opa1-mediated joining of the IMM (Fig. 3A)[24,25]. Opa1 has two isoforms: a long isoform (L-Opa1) containing a transmembrane domain, and a short isoform (S-Opa1) lacking the transmembrane domain. S-Opa1 is produced via proteolytic cleavage of L-Opa1 by one of two proteases, Yme1L or Oma1[26]. Yme1L knockdown produces a fragmented mitochondrial network, suggesting that Opa1 processing promotes fusion[26]. A separate study found that L-Opa1 was sufficient for fusion in cells lacking Yme1L and Oma1; conversely, S-Opa1 overexpression in these cells resulted in mitochondrial fragmentation[27]. These contrasting results raised the question of whether Opa1 processing promotes fission or fusion. Two recent studies used *in vitro* membrane fusion assays to gain mechanistic insight into Opa1-mediated fusion[28,29]. Both studies tested the sufficiency of Opa1 to facilitate membrane fusion of liposomes. In these assays, L-Opa1 is sufficient to

drive fusion through a heterotypic interaction with cardiolipin (CL), a mitochondrial phospholipid, whereas S-Opa1 is unable to drive fusion[28,29]. However, these studies found that S-Opa1 and L-Opa1 work synergistically to catalyze fusion. Ge *et al.* (2020) show that fusion efficiency peaks at an equimolar ratio of S-Opa1 to L-Opa1 (Fig. 3B)[28]. Thus, Opa1 processing tightly regulates fusion, with insufficient or excess processing inhibiting fusion.

Additional insight into the control of fusion has come from reexamination of Fis1 and mitochondria-ER contact sites. Mammalian Fis1 was initially thought to promote fission since its yeast homolog recruits Drp1 to mitochondria and because Fis1 overexpression induces mitochondrial fragmentation[30]. However, human Fis1 does not function through Drp1 and is dispensable for fission[3,5,6]. Fis1 has recently been shown to inhibit the activity of the fusion GTPases Opa1 and Mfn1/2[31], suggesting that fusion inhibition is sufficient to fragment the mitochondrial network, mirroring fission activation (Fig. 3C). Mitochondrial fusion also occurs at mitochondria-ER contact sites, similar to fission[22,32,33]. Fission and fusion proteins colocalize at mitochondria-ER contacts to form hotspots for membrane dynamics[32]; these ER-associated dynamics also include contact untethering between mitochondria[22]. Thus, the ER regulates multiple aspects of mitochondrial dynamics at contact sites. The next challenge is to determine how these separate machineries are coordinated to promote a single process.

Mitochondrial shape transition independent of fission/fusion

Mitochondrial shape varies depending on a variety of cellular signals. Two stimuli commonly used to alter mitochondrial network morphology are increased intracellular calcium and mitochondrial depolarization. Both produce a mitochondrial network comprised of small, rounded mitochondria, leading to speculation that both induce fission. However, Fung *et al.* (2019) revealed that calcium-induced and depolarization-induced mitochondrial fragmentation are distinct[34]. Calcium-induced actin assembly on mitochondria requires INF2, and thus undergoes canonical INF2-dependent fission (Fig. 2). Mitochondrial depolarization induced with the mitochondrial uncoupler carbonyl cyanide 3-chlorophenylhydrazone (CCCP) has a different effect on actin and mitochondria. CCCP-induced actin dynamics are INF2-independent, relying instead on the Arp2/3 complex to form transient actin clouds around depolarized mitochondria[34]. Depolarized mitochondria then undergo inner membrane rounding, resulting in shape deformation.

CCCP-induced mitochondrial deformations appear as rings. However, a recent study combining live imaging and volume electron microscopy found that mitochondrial rings are actually three-dimensional discs with central invaginations that only appear as rings in cross sections[35]. Most CCCP-induced shape changes were generated from rounding of the mitochondrion, rather than fission or fusion[34,35]. Consistently, CCCP-induced shape change is Drp1-independent and occurs due to IMM rearrangement, while the OMM remains intact[34]. Thus, CCCP-induced mitochondrial shape transition regulates the switch between a connected and fragmented mitochondrial network independent of fission/fusion (Fig. 3C).

Other mitochondrial inhibitors, such as inhibition of ATP synthesis with oligomycin, have distinct effects on mitochondrial morphology[9]. Thus, the relationship between mitochondrial

function and morphology depends on multiple factors; metabolic function affects mitochondrial dynamics through fission, fusion, transport, and more[36]. However, mitochondrial form does not always match function. In *Drosophila* neurons, normal mitochondrial function is required for organism viability, independent of mitochondrial distribution, indicating that these processes are separable[37].

Increasing cytosolic calcium has multiple effects on mitochondrial morphology. While increasing intracellular calcium promotes fission through canonical Drp1 oligomerization and actin polymerization[1,2,18], calcium separately affects mitochondrial morphology through Miro1, an OMM transmembrane protein with two GTPase domains and two calcium-binding EF hands. Calcium induces mitochondrial shortening by binding to a single EF-hand of Miro1[38]. Miro1-dependent mitochondrial shortening produces small, rounded mitochondria independent of Drp1, indicating this transition is distinct from fission[38]. Thus, calcium affects multiple aspects of mitochondrial morphology by promoting fission and shape transition through separate mechanisms. Additional work is needed to understand how these pathways intersect and cooperate to remodel the mitochondrial network in response to cytosolic calcium levels.

Mitochondrial transport and anchoring: stop and go on two cytoskeletons

Mitochondrial network morphology is also controlled by many additional interactions with the cytoskeleton. Mitochondrial transport is critical in highly polarized cells, such as neurons, where mitochondria undergo long-distance transport[39]. Most mitochondrial transport is microtubule-based, with transport toward the microtubule plus-end mediated by kinesin-1 and transport toward the minus-end mediated by cytoplasmic dynein 1 (dynein) and its partner complex, dynactin[40,41]. In the canonical model of mitochondrial transport, these opposing motors are tethered to mitochondria through the TRAK/Miro motor adaptor complex (Fig. 4A)[39,41]. TRAK1 and TRAK2, the mammalian orthologs of *Drosophila* Milton, interact with kinesin-1 and dynein-dynactin[41], while Miro1 and Miro2 function as calcium-sensitive adaptors that link the motor/TRAK complexes to mitochondria[42].

Motors, TRAKs and Miro proteins are required for mitochondrial transport, but the functional interactions among these components remain largely untested, and the molecular basis by which opposing kinesin and dynein motors are coordinated to produce directional transport of mitochondria is not understood. Motor regulation may be adaptor-specific. For instance, TRAK2 has been proposed to predominantly interact with dynein-dynactin whereas TRAK1 interacts with both kinesin-1 and dynein-dynactin[41]. TRAK1 overexpression promotes plus-end directed mitochondrial transport in mouse embryonic fibroblasts (MEFs) while TRAK2 overexpression promotes minus-end directed mitochondrial transport. However, TRAK2 requires Miro1, but not Miro2, to promote dynein-dependent transport[43]. Combined, these results suggest that the direction of mitochondrial transport is determined by specific associations between TRAK and Miro isoforms. Further studies are required to determine how individual TRAK and Miro proteins interact with microtubule motors to selectively promote transport toward either microtubule end.

A recent study found that TRAKs localize to mitochondria and drive transport in MEFs lacking Miro1 and Miro2[43]. While transport is reduced in Miro1/2 knockout cells, this finding suggests that TRAKs can function independently of Miro. Since TRAKs interact with other OMM proteins, such as Mfn1[44,45], another OMM protein may function as an alternate adaptor for TRAK1/2. Miro proteins also serve as adaptors for myosin XIX (Myo19), a mitochondria-associated myosin motor, though Myo19 can also associate with the OMM independent of Miro (Fig. 4A)[43,46,47]. Myo19 overexpression increases mitochondrial motility in an actin-dependent manner[48]. Furthermore, TRAK overexpression reduces the association of Myo19 with mitochondria[46], suggesting that Myo19 and TRAKs compete for Miro binding to induce actin- or microtubule-based mitochondrial motility. Given the nature of Miro proteins, it will be interesting to see how calcium binding and GTP hydrolysis affect the interaction of Miro with TRAKs and Myo19.

Mitochondria are also anchored to the cytoskeleton at specific cellular locations. Mitochondrial anchoring is particularly important in neurons, where mitochondria are tethered at presynaptic sites in axons to supply energy for neurotransmission. In mammalian neurons, actin stabilizes mitochondria at presynaptic terminals[49,50]. A recent study found that stationary mitochondria at presynaptic sites are more firmly anchored in place than other mitochondria[49]. The tethering of these presynaptic mitochondria is partially dependent on actin. Given that Myosin V and VI oppose mitochondrial motility in *Drosophila* neurons[51], and Myosin VI can form actin cages around mitochondria[52], it will be interesting to determine whether these myosins or a separate tether link mitochondria to actin for anchoring at presynaptic sites (Fig. 4B). Mitochondrial anchoring at presynaptic sites is also facilitated by syntaphilin, a microtubule-binding protein that has been proposed to dock mitochondria by binding kinesin-1, preventing motor activation by the Miro-TRAK complex (Fig. 4B)[53]. Thus, similar to mitochondrial transport, mitochondrial anchoring relies on both the actin and microtubule cytoskeletons and further investigations of mitochondrial anchoring must account for the effects of each.

Conclusion

As new imaging techniques have uncovered the precise shaping and remodeling of the mitochondrial network, we have increased our understanding of this dynamic organelle. Recent work has helped define the molecular dynamics of fission, fusion, shape transition, transport, and tethering. However, the mechanistic details of each process and their interplay with each other have not been worked out. The intersection of these pathways, with varied effects on mitochondrial morphology, gives rise to the morphological complexity found in this dynamic organelle. Future endeavors accounting for each aspect of mitochondrial dynamics will more fully uncover the nature of this organelle network and determine how it remodels and reshapes to fit cellular needs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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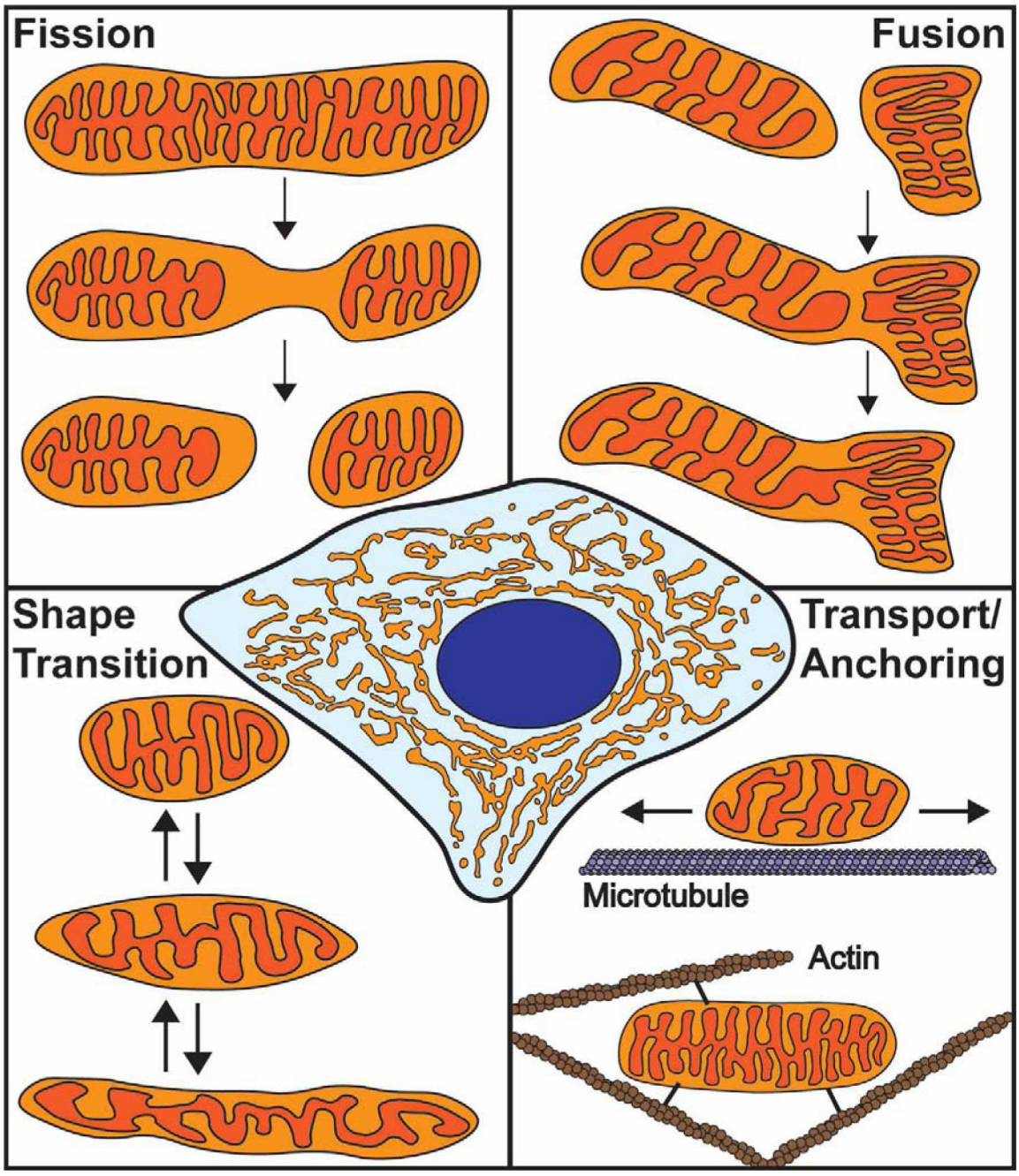

Figure 1. Overview of mitochondrial dynamics.

Mitochondria form a complex, interconnected network within the cell (center). The morphology of this network is determined by fission, fusion, mitochondrial shape transition, and positioning along the cytoskeleton. Fission begins with IMM division followed by OMM scission. Fusion involves merging of two outer membranes followed by joining of the inner membranes. Mitochondrial shape transition is a process independent of fission/fusion that controls the transition between rounded and elongated mitochondrial morphologies. Mitochondrial positioning involves transport and tethering along the microtubule and actin cytoskeletons.

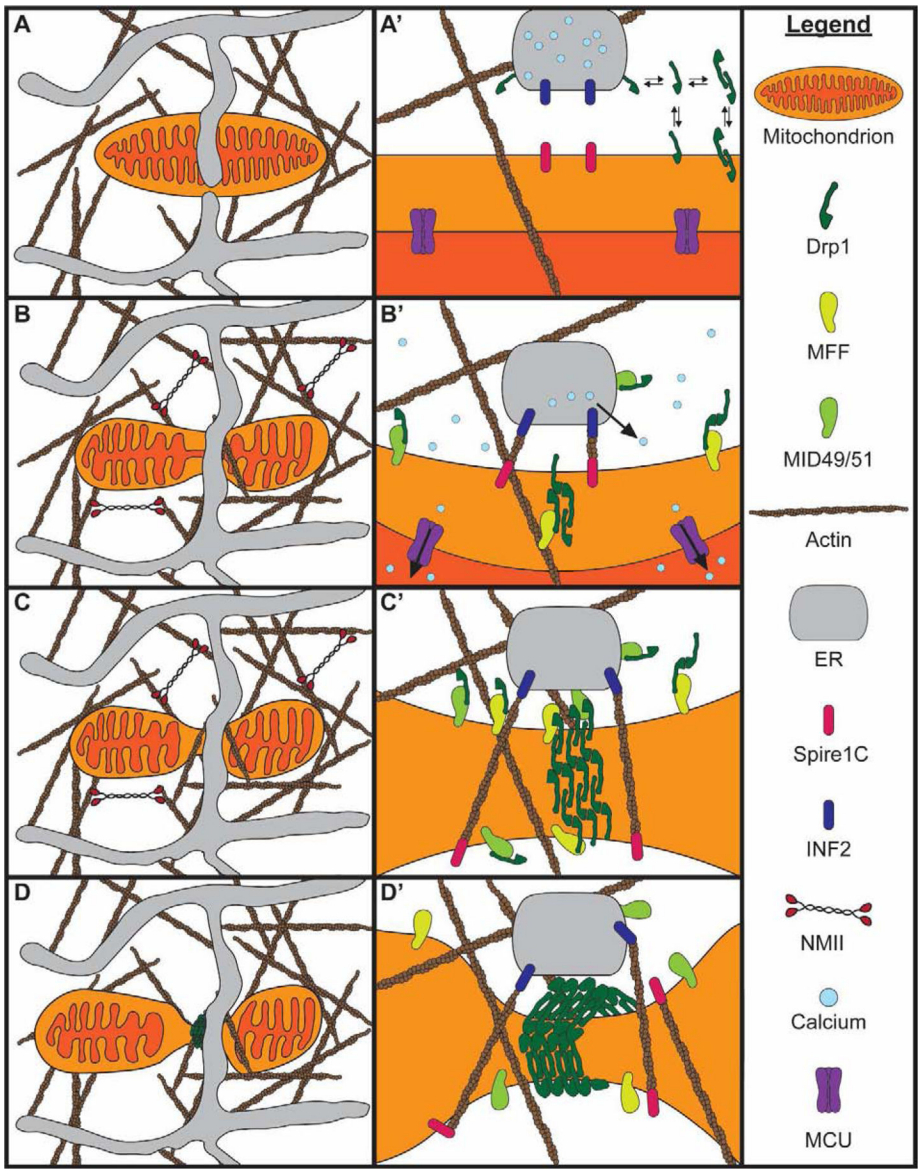

Figure 2. Mechanism of mitochondrial fission.

A) A mitochondrion is embedded in the interstitial actin network and closely associated with the ER. A closer view of the mitochondria-ER contact is shown in **A'**. Drp1 dynamically associates with the cytosol, mitochondria, and ER prior to fission. **B)** Peripheral NMII pulls on actin filaments to deform the mitochondrial membrane. Increased cytosolic calcium induces actin polymerization at mitochondria-ER contacts by INF2 on the ER and Spire1C on mitochondria. Mff and Mid49/51 begin recruiting Drp1 to the mitochondria-ER contact. Calcium is released from the ER and enters the mitochondria through the MCU, causing IMM constriction. **C)** Elevated mitochondrial matrix calcium causes IMM division prior to OMM division. Mff and Mid49/51 continue recruiting Drp1 to the mitochondria-ER contact, with some Drp1 coming from the ER. Drp1 oligomerizes along the constricted OMM. **D)** The Drp1 oligomer fully assembles around the OMM. Drp1 GTP hydrolysis dissociates

Mid49/51, constricting the Drp1 ring. The Drp1 ring constricts the OMM and completes the process of fission.

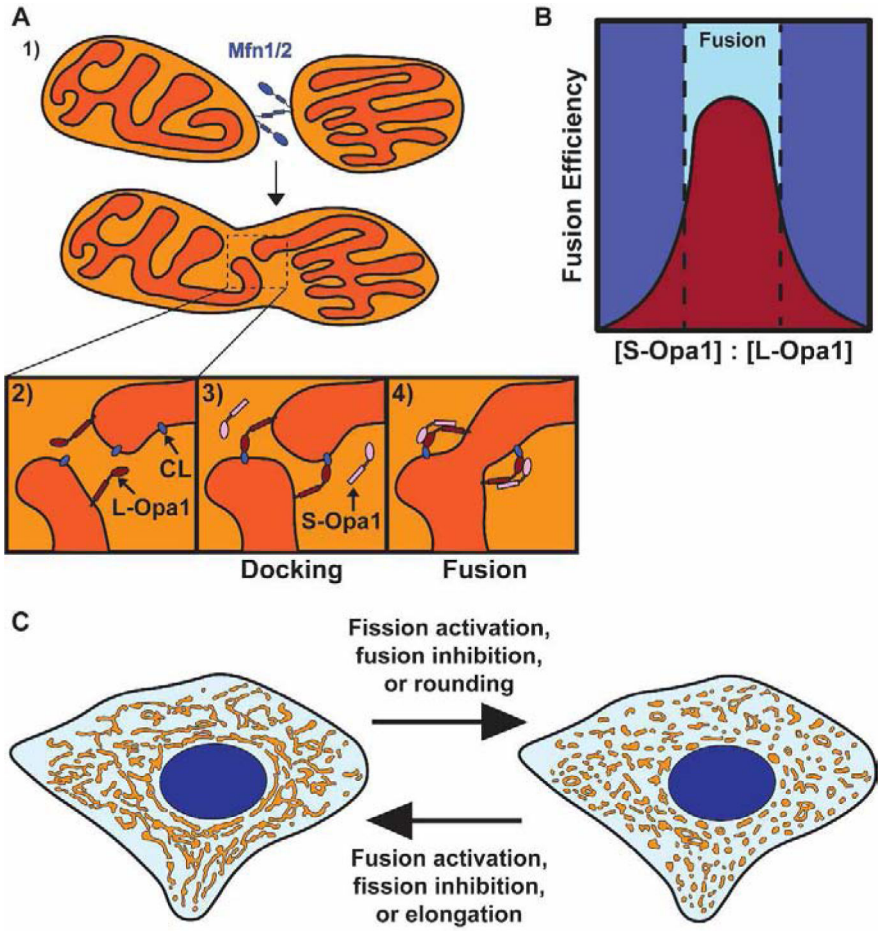

Figure 3. Mitochondrial fusion and shape transition.

A) Proposed model of mitochondrial fusion. 1) Mitochondrial fusion begins with Mfn1/2-mediated tethering of two mitochondrial outer membranes. 2) The inner membranes are positioned for fusion upon outer membrane fusion. 3) Interactions between L-Opa1 and cardiolipin (CL) dock the inner membranes, bringing them closer together. 4) S-Opa1 functions with L-Opa1 and cardiolipin to promote efficient inner membrane fusion. **B)** Fusion efficiency at different S-Opa1:L-Opa1 ratios. Fusion efficiency peaks at an equimolar ratio of S-Opa1 to L-Opa1, with higher and lower ratios inhibiting fusion. **C)** Schematic of a connected mitochondrial network (left) and fragmented mitochondrial network (right). The transition between these networks can occur through direct regulation of fission, fusion, or mitochondrial shape transition.

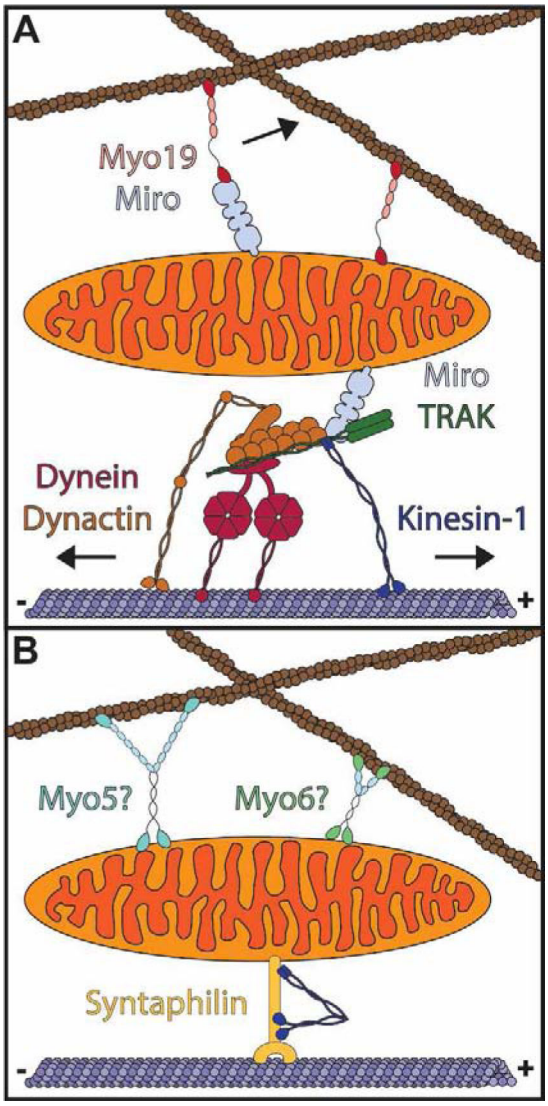

Figure 4. Mitochondrial transport and anchoring on the cytoskeleton.

A) TRAK and Miro proteins serve as adaptors for microtubule-based mitochondrial transport (below). Kinesin-1 drives transport to the microtubule plus-end while transport to the microtubule minus end is mediated by dynein/dynactin. Myo19 associates with Miro proteins and directly with the mitochondrial outer membrane to drive mitochondrial transport along the actin cytoskeleton. **B)** Mitochondria are anchored to the actin and microtubule cytoskeletons. Syntaphilin anchors mitochondria to microtubules while myosin V (Myo5), myosin VI (Myo6), or another tether may anchor mitochondria to actin.