Review

Function and regulation of the divisome for mitochondrial fission

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Mitochondria form dynamic networks in the cell that are balanced by the flux of iterative fusion and fission events of the organelles. It is now appreciated that mitochondrial fission also represents an end-point event in a signalling axis that allows cells to sense and respond to external cues. The fission process is orchestrated by membrane-associated adaptors, influenced by organellar and cytoskeletal interactions and ultimately executed by the dynamin-like GTPase DRP1. Here we invoke the framework of the 'mitochondrial divisome', which is conceptually and operationally similar to the bacterial cell-division machinery. We review the functional and regulatory aspects of the mitochondrial divisome and, within this framework, parse the core from the accessory machinery. In so doing, we transition from a phenomenological to a mechanistic understanding of the fission process.

Over 100 years ago, Margaret Reed Lewis and Warren Lewis published their microscopic observations of dynamic mitochondrial networks in living chick fibroblasts[1]. They reported that mitochondria displayed all sorts of shapes and noted that mitochondrial 'rods or threads may change into granules, threads may fuse or branch into networks' and that 'degenerating mitochondria may separate into granules and vesicles'. These seminal observations have stood the test of time and in the past two decades, much focus has been placed on the relationship between mitochondrial form and function and on the machinery that controls mitochondrial shape. Mitochondria are symbiotically derived, double-membrane organelles of eukaryotic cells and have a plethora of functions, including oxidative phosphorylation (OXPHOS) for ATP production, biosynthesis of Fe–S clusters, haem, nucleotides and amino acids and also Ca^{2+} homeostasis[2]. In contrast to other organelles (such as lysosomes and peroxisomes), mitochondria cannot be formed de novo. They still contain DNA (mitochondrial (mt)DNA) that must be replicated and inherited for cells to respire. Mitochondria therefore have to be generated from pre-existing organelles and mitochondrial growth is thereby fundamentally linked to fission[3] (Fig. 1). Dedicated protein machineries are responsible for the fission and fusion of mitochondria, which allows for efficient organelle distribution during cell division and the mixing of organelles for repair or as an adaptation to environmental stimuli[4–7]. This review focuses on the machinery involved in mitochondrial fission and how cytoplasmic factors control this once-independent replicating structure. Several proteins are involved in this process and none of them is sufficient. We therefore introduce the concept of the 'mitochondrial divisome', akin to the bacterial division machinery[8] (Fig. 2).

Evolution of the mitochondrial divisome

According to the endosymbiotic theory, mitochondria descended from a free-living alphaproteobacterium that was engulfed by a host cell possibly related to the Asgard archaea[9]. In bacteria, division through binary fission manifests as a coordinated process that involves a dozen conserved proteins that together form the 'divisome'[10,11] (Fig. 2). The placement of the divisome is determined by the Min system, which antagonizes the assembly of the bacterial tubulin homologue FtsZ at the cell poles. Tethered by inner-membrane-anchored proteins (FtsA and ZipA), FtsZ forms a circumferential ring at the middle of the cell, which constricts the bacterial cell. This FtsZ ring acts as a scaffold to coordinate the local deposition of nascent peptidoglycan or septum at the site of constriction on the outside of the membrane, through its interactions with other proteins (FtsW, FtsI and FtsN). Inside, the FtsZ ring interacts with the DNA translocase FtsK, which helps to resolve and remove the chromosome from the constricting septum. The septum is essential for constriction, possibly by acting as a Brownian ratchet to peg incremental degrees of membrane bending forced by the FtsZ ring. Although the FtsZ ring is often referred to as the primary force-generating machinery that constricts the bacterial cell, its ability to convert energy from GTP hydrolysis to drive cell division thus relies on inputs from numerous additional proteins.

Over the approximately 1–2 billion years of eukaryote evolution, most of the bacterial genome was lost with a few genes transferred to the host nuclear genome. Subsequent evolution—under constraints imposed by an endosymbiotic relationship—transformed the bacterium into an organelle with two notable topological features: (a) a folded inner membrane of prokaryotic origin that is bordered by an outer membrane derived from the host-cell plasma membrane; and (b) the capacity to undergo homotypic fusion, resulting in the formation of a continuous network. These changes led to substantial evolution of the divisome, with mitochondrial fission now requiring the priming of a constriction site on the outer membrane through inputs from cytoskeletal elements and interactions with other organelles, and subsequent fission by host-genome-encoded proteins of the dynamin family[12]. We can further appreciate such evolution through findings from extant organisms that use both the prokaryotic divisome and eukaryotic dynamins for mitochondrial fission. In the red alga *Cyanidioschyzon*

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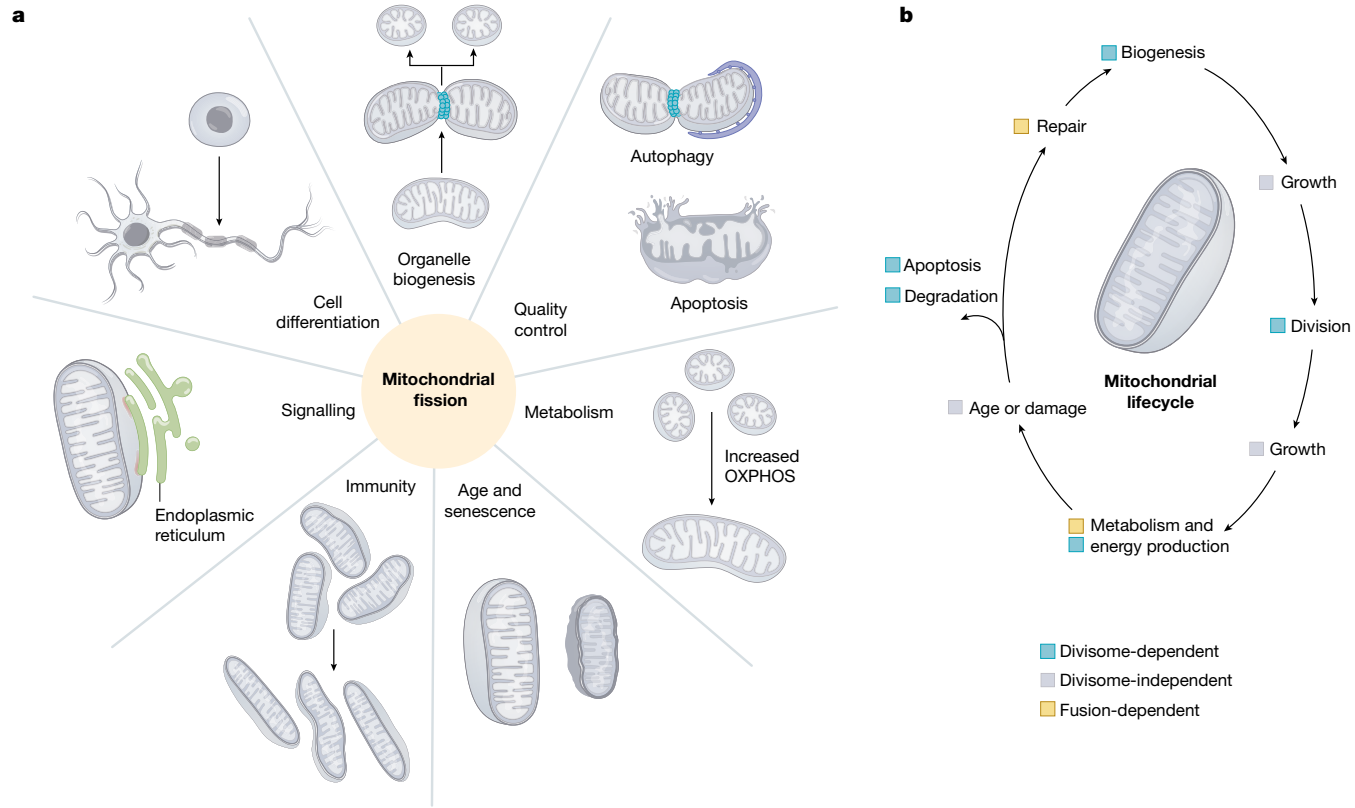

Fig. 1 | Cellular and physiological importance of the mitochondrial divisome. a, Mitochondrial fission alters the global mitochondrial network and affects a variety of cellular processes. **b**, Steps in a mitochondrial lifecycle that necessitate mitochondrial fission and fusion processes.

merolae, the mitochondrial FtsZ protein partially constricts the organelle, which enables the dynamin homologue Dnm1 to assemble as cytoplasmic patches to finally sever the organelle[8,13]. Thus, a distinct upstream mechanism serves to both define the site of fission as well as to partially remodel organelle topology before dynamin takes over to complete the fission process.

In many eukaryotes (including yeasts and animals), mitochondria form elaborate and dynamic tubular networks. In these organisms, mitochondria divide by a process similar to binary fission with homeostatic control of growth and—in some cases—nucleoid partitioning, akin to the programmed DNA replication and cell division processes seen in bacteria (Fig. 2). Notably, however, the divisome functions in the complete absence of the contractile FtsZ ring apparatus.

Core components of the eukaryotic divisome

Dynamin-family proteins are required for executing fission from the outer face of the organelle. Divisome assembly is managed by a set of conserved integral membrane proteins or adaptors, some of which are organized at sites of mitochondrial fission. We categorize these as core components of the divisome.

Dynamin-related protein 1

The executioner of mitochondrial fission is the GTPase of the dynamin superfamily of proteins, known as dynamin-related protein 1 (DRP1). Once recruited to the outer mitochondrial membrane, DRP1 forms helical oligomers that induce membrane constriction and severing. Loss of DRP1 results in highly elongated mitochondria, as a result of unopposed fusion events[14–18]. DRP1 also divides peroxisomes: elongated peroxisomes are seen upon loss of DRP1, as peroxisomes grow[14,19].

Dynamin-superfamily proteins (DSPs) have evolved to manage membrane fission or fusion reactions. In broad categories, DSPs with a transmembrane domain function in membrane fusion, whereas soluble DSPs manage membrane fission. All fission DSPs have a similar architecture, in which the polypeptide chain folds back on itself to give rise to a monomer with four domains (the head, neck, trunk and foot of the molecule) (Fig. 3). The GTP-binding G domain represents the head of the molecule. The stalk domain forms the trunk of the molecule and contains important interfaces for self-assembly[20,21]. The head and trunk are connected by a neck, the bundle signalling element. Flexible hinges allow substantial degrees of freedom of the head and trunk domains relative to the neck[20–26]. Interactions with adaptors on the mitochondrial outer membrane occur at the G domain and the stalk[20]. In DRP1, the foot represents an approximately 100-residue-long unstructured region known as the variable domain, which binds negatively charged lipids such as cardiolipin and phosphatidic acid[27–30]. DSPs form an X-shaped dimer through a large and highly conserved hydrophobic patch (interface 2) that is present at the centre of the trunk. Interactions across interface 1 (which are present on the trunk, towards the head) form tetramers. Interactions across interface 3 (which are present on the trunk, towards the foot) facilitate the self-assembly of tetramers into higher-order oligomers. The variable domain prevents premature self-assembly, probably by capping interface 3[20,21,31–33].

DRP1 adaptors

In cells, DRP1 requires specific adaptor proteins to engage with the mitochondrial outer membrane. Adaptors are as integral to the divisome as is DRP1 itself, because (a) the loss of adaptors largely phenocopies the loss of DRP1 (in leading to defects in mitochondrial fission), (b) adaptor interactions differentially regulate the self-assembly and catalytic activities of DRP1 and (c) cues that mark the assembly of the fission apparatus may lie in membrane organization of the adaptors.

In yeast, the tail-anchored mitochondrial outer-membrane protein known as Fis1 acts as an adaptor for the yeast-specific peripheral

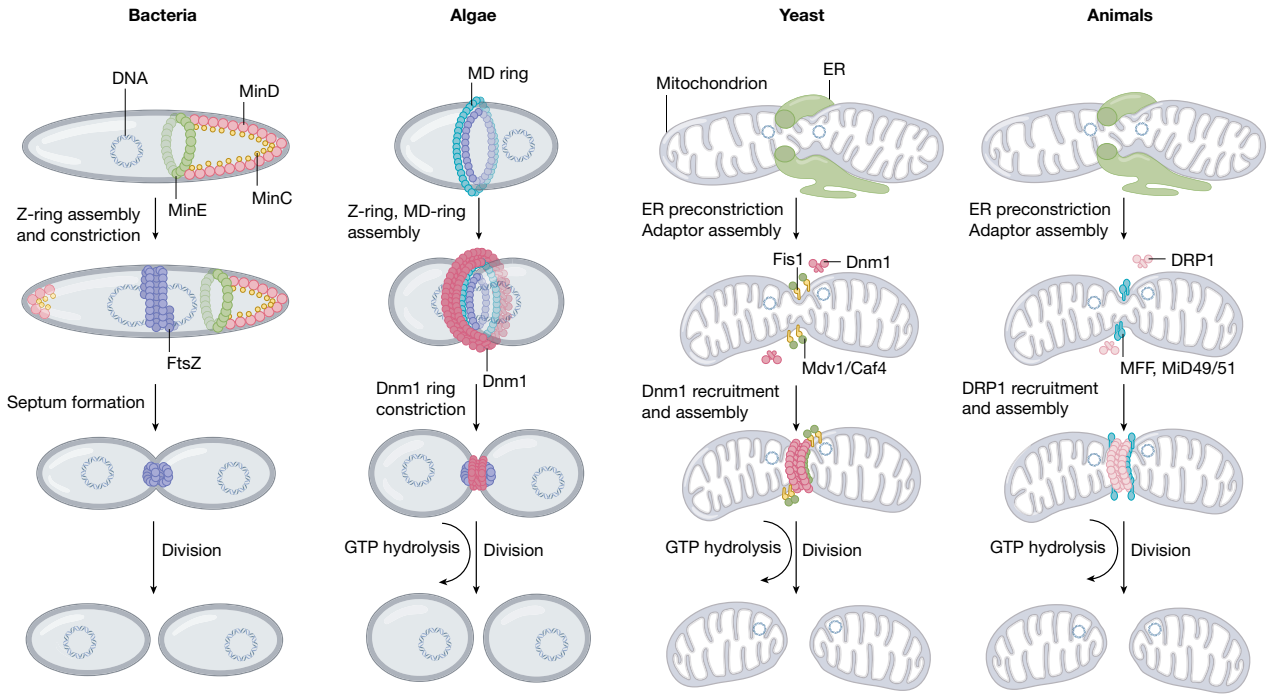

Fig. 2 | Evolution of the divisome. Bacterial, algal (*C. merolae*) and the eukaryotic divisome machineries, emphasizing their core components. Division is a stepwise process that involves assembly of a contractile ring at the membrane and subsequent fission. In bacteria, the divisome is a multicomponent system, in which FtsZ forms the contractile ring. FtsZ assembly is regulated by MinC, MinD and MinE. The FtsZ ring facilitates septum formation on the outside of the membrane, as well as segregation of chromosomes. In the red alga *C. merolae*, a mitochondrial FtsZ protein partially constricts the organelle, which enables the dynamin homologue Dnm1 to assemble with the mitochondrion-dividing (MD) ring on the cytosolic face to induce fission. In many eukaryotes (including yeasts and animals), the divisome functions in the complete absence of the contractile FtsZ ring apparatus. Constriction is induced by the endoplasmic reticulum (ER), actin and other factors, allowing activation and/or assembly of membrane adaptors (Fis1 with Mdv1 or Caf4 in yeast; MFF, MiD49 or MiD51 in animals) and recruitment of the dynamin-related protein (Dnm1 in yeast; DRP1 in animals) that acts as the membrane constrictase.

membrane proteins Mdv1 and Caf4, which then recruit and assemble with the yeast DRP1 homologue, Dnm1[34,35]. Metazoans lack homologues of Mdv1 or Caf4, and Fis1 does not appear to be required for mitochondrial fission in mammalian cells[36,37], plants[38] or apicomplexan parasites[39]. Mammalian FIS1 has instead been implicated as an adaptor for proteins that are involved in the selective autophagy of mitochondria (mitophagy)[40,41]. Metazoans have evolved other adaptors that recruit DRP1. These include the mitochondrial fission factor (MFF), along with the chordate-specific mitochondrial dynamics proteins of 49 kDa (MiD49 (also known as MIEF2)) and 51 kDa (MiD51 (also known as MIEF1)). MFF is a tail-anchored protein that integrates into the mitochondrial outer membrane and peroxisomal membrane, and which serves as the general fission adaptor[42,43]. MiD49 and MiD51 have inactive nucleotidyltransferase folds with an N-terminal transmembrane anchor for specific integration into the mitochondrial outer membrane[36,44–49]. Each adaptor can independently recruit DRP1 to mitochondria and their loss causes defects in mitochondrial fission[34,36,37,44,46,47,50–53].

Evidence exists that adaptors can show a clustered organization on the outer mitochondrial membrane, where they engage with DRP1 during fission[36,37,49,54,55]. In the absence of DRP1, MiD49 and MiD51 become diffuse in organization. By contrast, MFF can directly cluster at mechanically induced constrictions on the mitochondrion[56], which suggests that determinants for MFF clustering are intrinsic to MFF itself (for example, via its transmembrane anchor and/or through oligomerization)[37,44,49]

The fine-tuning of core components

Alternate splicing of the *DNM1L* gene (which encodes DRP1) produces at least ten different isoforms that display tissue-specific distribution patterns and activity[57–59]. Furthermore, post-translational modifications (phosphorylation, ubiquitylation, *S*-nitrosylation and sumoylation) can occur exclusively or concurrently on the DRP1 molecule, allowing for the further regulation of mitochondrial fission[60,61]. Two phosphorylation sites on DRP1 (Ser616 and Ser637) are currently best understood. Phosphorylation of Ser616 stimulates DRP1 activity and leads to increased mitochondrial fission, whereas phosphorylation of Ser637 inhibits fission[60,62]. Protein kinase A (PKA)-induced phosphorylation of Ser637 dampens the GTPase activity of DRP1, whereas dephosphorylation via Ca^{2+}-activated calcineurin triggers fission[63,64]. The mitochondrial phosphatase (phosphoglycerate mutase 5 (PGAM5)) is also involved in Ser637 dephosphorylation and its loss impairs mitochondrial fission and increases cellular senescence[65].

Adaptors are also subject to post-translational modifications that alter fission activity. The mitochondrial E3 ubiquitin ligase MARCH5 regulates levels of MiD49 and therefore DRP1 assembly, but is itself regulated by MFF and DRP1[66,67]. Cellular stress and metabolic status have been linked to the phosphorylation of MFF via AMP-activated protein kinase (AMPK), thereby integrating mitochondrial fission into a wider cellular context[68] (as discussed in 'Metabolic state').

Accessory components of the divisome

Here we categorize factors that modulate the timing, frequency and positioning of mitochondrial fission—actin, interorganelle contact sites and specific lipids displayed on the outer membrane—as accessory components of the divisome. This includes contacts with other organelles such as the ER and lysosomes, as well as the actin- and cytoskeleton-related binding proteins inverted formin 2 (INF2)

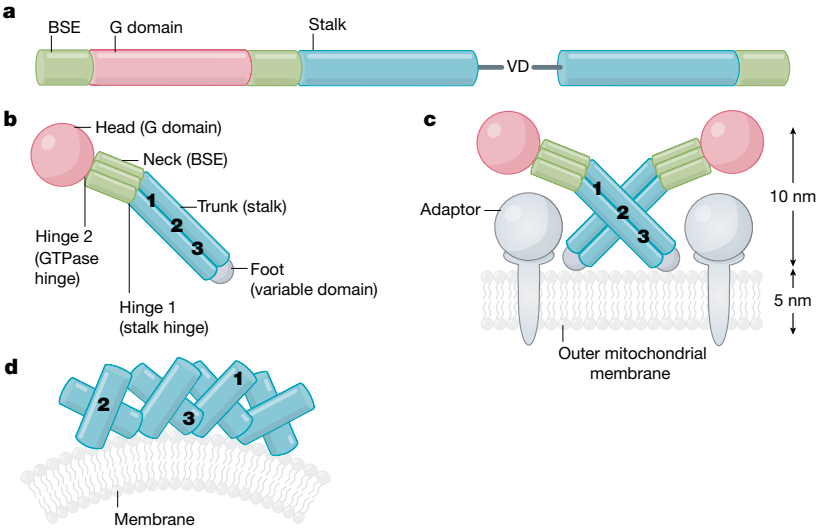

Fig. 3 | Structure and organization of DRP1. a, Domain organization of DRP1. BSE, bundle signalling element; VD, variable domain. **b**, Organization of domains in the folded DRP1 monomer, defining four regions in the molecule: the head (G domain), the neck (formed by three BSE helices coming together), the trunk (formed by two stalk helices coming together) and the foot (formed by the variable domain). Interfaces 1, 2 and 3 on the trunk determine self-assembly; monomers bind one another to form a dimer through interface 2; dimers bind one another through interface 1; and oligomerization on the membrane proceeds through interactions through interface 3. **c**, Presumed organization of DRP1 on the mitochondrial outer membrane engaged with adaptor proteins. **d**, Self-assembled DRP1 on the membrane with the relevant interfaces marked. Only the trunk of the molecule is shown, for clarity. In solution, interfaces 1 and 2 are accessible; they ensure that DRP1 exists in a dimer–tetramer equilibrium. On the membrane, the unmasking of interface 3 results in oligomerization.

and SPIRE1C (Fig. 4). Although the core divisome is able to cut lipid tubes in vitro, these accessory components are required for initiating and controlling mitochondrial fission in the complex environment of living cells. Interorganelle contacts with mitochondria have been reported over many decades, but only recently has their functional importance come to light with the identification of various tethering machineries[69,70]. The region of the ER that is in close proximity to mitochondria is known as the mitochondria-associated membrane. Mitochondria-associated membranes are enriched in proteins for a range of key cellular functions, including phospholipid biosynthesis, lipid exchange and Ca^{2+} and reactive oxygen species signalling[71,72]. In addition, ER tubules function to preconstrict mitochondria in the early stages of fission[55]. In fact, the majority (about 90%) of mitochondrial fission events are spatially associated with ER tubules—these events are therefore referred to as ER-associated mitochondrial divisions, even though only a small fraction (about 10%) of these contacts actually coincide with mitochondrial constriction and an even smaller fraction (around 1%) undergo fission[55]. This points to the coordinated involvement of additional factors at the contacts to prime mitochondrial fission.

The cytoskeleton is a crucial player in regulating organellar morphology. In particular, the actin cytoskeleton is involved in mitochondrial fission and often uses the ER as a platform for initiating constriction events. Inhibiting actin polymerization with latrunculin B causes mitochondrial elongation[73]. Moreover, transient actin polymerization on a subset of mitochondrial populations is associated with fission[74]. These transient polymerization events appear to be governed by mitochondrial length, thus maintaining a balanced organellar network. An ER-localized isoform of INF2 causes actin polymerization along mitochondria with the help of the mitochondria-localized actin nucleator SPIRE1C[75,76]. Actin polymerization at the interorganelle contact site causes mitochondrial constrictions, which enable the activation of the core fission machinery. It has been found that INF2-mediated actin polymerization results in an increase of Ca^{2+} flux from the ER to mitochondria at organelle contact sites, which ultimately results in constriction and eventual scission of the inner mitochondrial membrane before organelle fission[77]. Even though actin assembly occurs immediately before fission, how a polymerizing actin network is able to constrict mitochondria is not completely understood: it may involve the motor protein myosin II. Ultrastructure analysis of cells shows that actin bundles are held diagonally to one another at (or near) the site of constriction and are associated with non-muscle myosin II[78]. Inhibition or depletion of myosin II reduces DRP1 binding to mitochondria and increases mitochondrial length[79]. The criss-cross arrangement of actin at the contact site with the help of non-muscle myosin II may generate localized force on the mitochondrion, which leads to constriction and subsequent assembly of the fission apparatus. Notably, direct assembly of DRP1 oligomers on actin filaments has also been reported[80], which may allow a population of 'primed' DRP1 to assemble quickly at fission sites.

In addition to the established and important role of the ER in the divisome, both lysosome and the *trans*-Golgi network contacts with mitochondria have also been implicated in fission. Lysosomes may associate at mitochondrial constriction sites before fission[81]. Mechanistically, the mitochondria-localized GTPase-activating protein TBC1D15 engages with lysosomal-associated RAB7. After GTP hydrolysis, RAB7 dissociates from the lysosome, which leads to the untethering of lysosome–mitochondria contacts and allows mitochondria fission. It has recently been found that mitochondrial fission involves the delivery of phosphatidylinositol 4-phosphate (PI(4)P) from *trans*-Golgi vesicles to ER–mitochondria contact sites[82]. ARF1—a small GTPase that regulates membrane dynamics of the secretory pathway—also has a role at this step[83], by regulating PI(4)P production through phosphatidylinositol 4-kinase IIIβ (PI4Kβ). ARF1 and PI(4)P *trans*-Golgi vesicles colocalize at DRP1-positive mitochondrial fission sites that are also marked by lysosomes. Depletion of ARF1 or PI4Kβ increases mitochondrial length, but not DRP1 recruitment; this suggests that the loss of PI(4)P stalls the late stages of DRP1-mediated fission[84]. Thus, a multitude of interorganelle contacts have a crucial role in determining mitochondrial architecture by modulating fission events. This may be fundamentally important for the dynamic organization of cellular compartmentation.

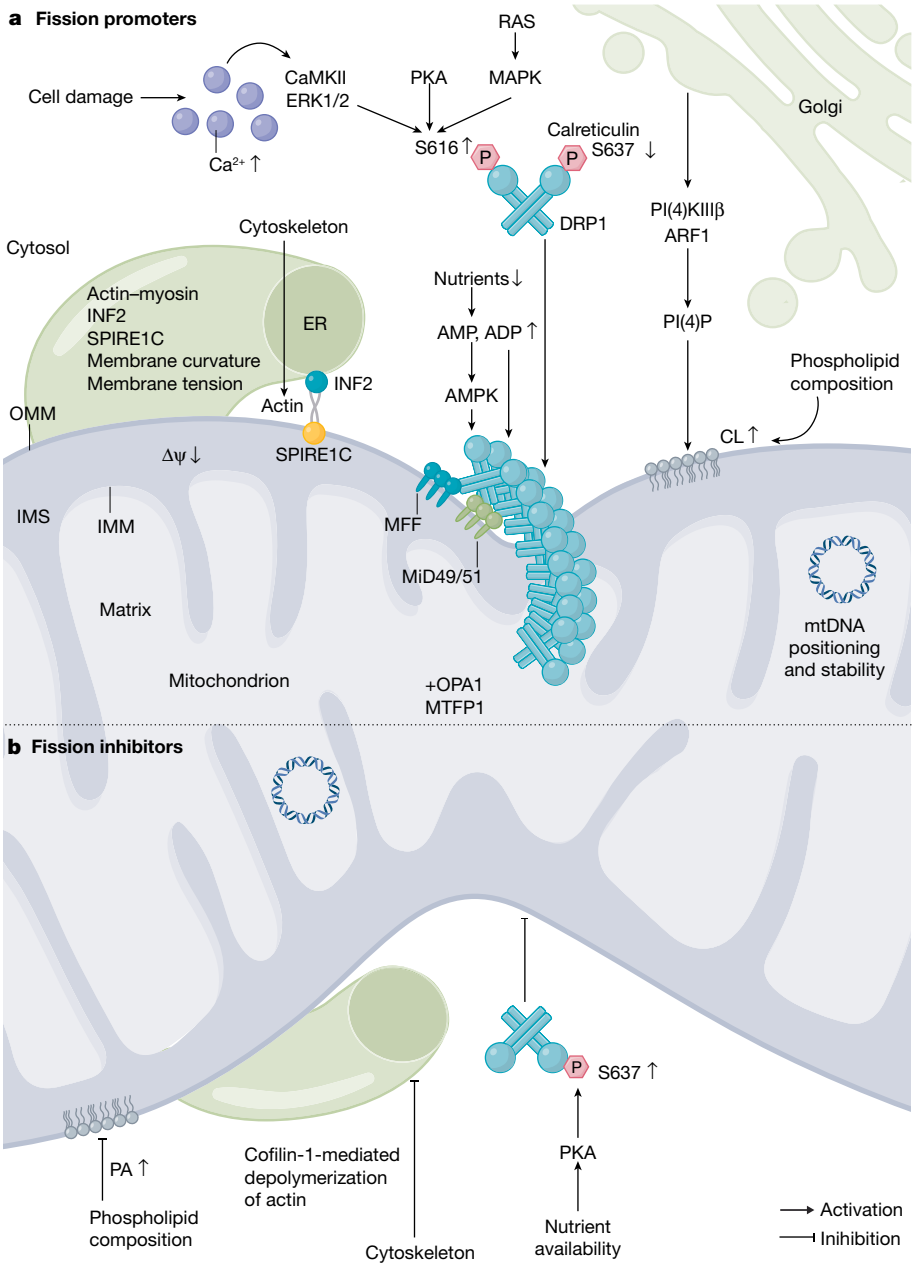

Fig. 4 | Regulation of the mitochondrial divisome. A range of cellular signals, with both chemical and mechanistic manifestations, regulate the core components of the divisome. These include changes in phospholipid composition, remodelling of the cellular cytoskeleton and local contacts with other organelles (including the ER, lysosomes and Golgi-derived vesicles). The divisome is also regulated by post-translational modification of the core constrictase, which is governed by signalling cascades that respond to metabolic cues. **a**, Fission-enhancing factors. **b**, Fission suppressors. CaMKII, Ca^{2+}/calmodulin-dependent protein kinase; CL, cardiolipin; ERK1/2, extracellular signal-regulated protein kinases 1 and 2; IMM, inner mitochondrial membrane; IMS, intermembrane space; MAPK, mitogen-activated protein kinase; OMM, outer mitochondrial membrane; P, phosphorylation site; PA, phosphatidic acid; Δψ, membrane potential.

Fission of the inner mitochondrial compartment

How the core and accessory components of the mitochondrial divisome are linked to the internal workings of the organelle remains an open question. Studies have shown that mtDNA replication by the mitochondrial polymerase POLG2 is spatially linked with ER-positive mitochondrial fission sites[84]. Conceivably, mtDNA replication could be synchronized with fission to ensure the correct segregation of the genetic material. However, factors that govern such intraorganelle communication are currently unknown. Conversely, spatial linkage between mtDNA and fission sites could reflect the underlying mitochondrial ultrastructure and the energetic constraints imposed. Live-cell stimulated emission depletion microscopy of mitochondria has revealed that mtDNA occupies discrete foci in between voids in the cristae[85]. These voids would represent regions with the lowest resistance for the divisome to assemble and constrict the organelle. Additional intramitochondrial proteins may facilitate fission of the inner compartment. Optic atrophy protein 1 (OPA1) is a mitochondria-localized DSP that is essential for inner-mitochondrial-membrane fusion via its membrane-anchored long isoform (L-OPA1)[86,87]. Processing of OPA1 by the stress-activated protease OMA1 generates a short isoform (S-OPA1) that facilitates fission of the inner mitochondrial compartment[88]. Two recently published studies have revealed the structures of both isoforms of OPA1, which shed light on how OPA1 executes its

action[89,90]. Although it is structurally similar to other DSPs, the stalk in Mgm1 (the yeast homologue of OPA1) is highly 'kinked'[89,90]. When added during liposome formation, OPA1 was able to oligomerize into helical assemblies both outside and inside of the liposomes. On the basis of its orientation vis-à-vis the membrane, this study suggested that OPA1 assemblies could either bulge or constrict the liposome in response to GTP-driven conformational changes[89]. Furthermore, it has also been suggested that inner-membrane constriction occurs independently of outer-membrane constriction and represents a priming event for mitochondrial fission[91]. Live-cell microscopy has shown that these oscillating inner-compartment constrictions coincide with ER–mitochondria contact sites. Consistent with previous findings, remodelling of mitochondrial cristae by S-OPA1 regulates inner-membrane constrictions, which highlights the dual role of OPA1 in membrane dynamics[91]. Assembly of DRP1 with MiD49 or MiD51—but not MFF—influences OPA1-mediated remodelling of cristae for release of cytochrome *c* during intrinsic apoptosis, thus representing cross-talk between divisome assembly on the mitochondrial outer membrane and remodelling of the mitochondrial inner membrane[44]. Such communication could be mediated by Ca^{2+} influx through interorganelle contact sites. Ca^{2+} entry could facilitate cristae reorganization, probably by affecting OPA1 function[91] and/or by promoting the cardiolipin-rich inner mitochondrial membrane to undergo a lamellar to nonlamellar phase transition[92]. Together, these reports further emphasize how a coordinated assembly of the divisome facilitates remodelling of both the outer and the inner mitochondrial membranes.

Mechanistic principles of divisome function

Molecular interactions between the core components alone may be informative for how the divisome is orchestrated on the mitochondrial outer membrane. DRP1 binds lipids and membrane-localized adaptors. Accordingly, at physiological concentrations of protein, DRP1 preferentially self-assembles on the membrane rather than in solution because the membrane acts as a concentrating device that allows it to attain the critical nucleation concentration at lower total protein concentrations. The binding of the variable domain with the membrane or engagement with membrane adaptors could also facilitate self-assembly[20,21,32]. The tendency of DRP1 to self-assemble into oligomers with an intrinsic curvature means that it preferentially binds and senses positive membrane curvature. However, the spontaneous self-assembly of DRP1 necessitates an energetically demanding process or mechanism for its disassembly. Thus, factors that affect the nucleation and subsequent disassembly for recycling become critical regulators of divisome function.

Previous electron microscopy analysis of the algal Dnm1 suggests a pathway that involves tetramers that self-assemble into short crescent-shaped filaments and eventually into ordered rings of about 40 nm in diameter[24]. In the presence of nonhydrolysable GTP analogues, the yeast and mammalian DRPs form right-handed spirals with an average outer diameter of around 100 nm and around 35 nm, respectively, which convert into rings with GTP[93,94]. Rings formed with mammalian DRP1 are typically composed of about 16 monomers with a height of around 10 nm, and an outer and inner diameter of about 30 nm and about 20 nm, respectively[93]. These assemblies therefore represent a highly curved state of the polymer, which—if organized around a membrane tube—would render a lumen of 10 nm in diameter. Self-assembly into spirals causes inter-rung interactions between G domains (G–G interactions) that reposition catalytic residues, which causes an increase in the rates of GTP hydrolysis[95]. DRP1, as with other dynamins, uses the energy from GTP hydrolysis to undertake conformational changes, thereby exerting a 'power-stroke' to effect constriction of the underlying membrane tube[96]. However, recent cryo-electron microscopy studies suggest an alternative pathway in which linear cofilaments of DRP1 dissociated from the MiD proteins were seen to curl in the presence of GTP[20], suggesting a mechanism for constriction. In vitro reconstitution experiments have enabled understanding of the scission capacity of the core machinery[97,98]. Using supported membrane tube assays with membranes containing high concentrations of cardiolipin, DRP1 could readily sever lipid tubes of sizes up to 400 nm in diameter. As mitochondrial diameters fall in the 200–300-nm size range[18], this meets the capacity of mitochondrial scission. By contrast, the ubiquitous endocytic dynamin 2 (DNM2) managed to sever tubes only of sizes below 40 nm[14], consistent with it acting on the narrow necks of endocytic pits[99]. These two DSPs therefore appear to have evolved mechanisms to cope with constraints imposed by organelle size. Lineage tracing of DSPs indicates that present-day mitochondrial and endocytic DSPs evolved from an ancestral bifunctional fission dynamin that managed both mitochondrial fission and vesicle release[100]. DNM2 has been implicated in executing the final membrane scission event required for mitochondrial fission[101]. Knockdown of *DNM2* mediated by small interfering RNA showed elongated mitochondria, in which DRP1 was arrested around constriction necks. However, the requirement of DNM2 in mitochondrial fission has been challenged by a number of studies[14,82,102]. Neither the loss of DNM2 nor all of the conventional dynamins (DNM1, DNM2 and DNM3) led to an inhibition of mitochondrial and peroxisomal fission, which suggests that DRP1 is sufficient for fission of both organelles. The demonstration that DRP1 can, on its own, sever membrane tubules supports these findings[14]. However, DMN2 may have a nonessential role in fission, given that it has been observed at mitochondrial fission sites flanked by DRP1[14,101].

Mitochondrial fission in health and disease

There are several cellular consequences if mitochondrial fission is disrupted. Cultured mammalian cells can survive without DRP1, and hence without mitochondrial fission. The fused mitochondrial network is still inherited by daughter cells during mitosis, perhaps through shear stresses generated during cytokinesis that act as a surrogate for DRP1 action (although segregation can be unequal)[16]. The importance of mitochondrial fission is more clearly seen in animal studies and disease states in which the fused and aggregated mitochondrial network impairs cell organization and impinges on a variety of processes, including ER contacts, Ca^{2+} signalling, autophagy and apoptosis. For example, DRP1-knockout mice are embryonic lethal: embryos display defects in forebrain maturation, defective synapse formation in neurons, compromised cardiac function and neural tube defects[16,17]. Tissue-specific knockout of DRP1 in the cerebellum[17] or muscle[103] also causes lethality. Depletion of DRP1 in adult mice results in muscle atrophy and degeneration, with swollen mitochondria and reduced respiration that is attributed to poor Ca^{2+} handling, blocked autophagy and myofibre death[103]. Pancreatic β-cells that lack DRP1 show Ca^{2+}-handling defects and impaired glucose-stimulated insulin secretion[104]. The loss of DRP1 in T cells leads to defects in T cell maturation, proliferation, metabolic reprogramming and antitumour responses[105]. The existence of these phenotypes in a wide variety of differentiated cell types highlights the importance of maintaining a dynamic mitochondrial network that involves fission. Nevertheless, concomitantly inhibiting fission and fusion can suppress cellular dysfunction, including in yeast[106], cardiac tissue[107] and hepatocytes[108].

Pathologies of divisome dysfunction

In humans, mutations in *DNM1L* lead to debilitating disease and—most often—death within early infancy (Table 1). A number of de novo dominant-negative mutations in DRP1 have been identified as impairing assembly or GTPase activity, and result in infantile epileptic encephalopathy[109,110]. Individuals may also present with optic atrophy, developmental delay, microcephaly, hypotonia and lactic acidosis—features that are typically seen in individuals with mitochondrial disease owing to defects in ATP generation[111]. Mutations in MFF also lead to mitochondrial

Table 1 | Mitochondrial divisome in health and disease

Function	DRP1	Study	Adaptor proteins	Study
Brain and central nervous system	Neurotransmission	Refs. [137,140,141]	Neurotransmission	Ref. [142]
	Neuroprotection and development	Refs. [16,17,143]	Encephalopathy	Ref. [112]
	Neurodegeneration	Refs. [144]	Myopathy	Ref. [113]
	Encephalopathy	Refs. [109,110]	Optic atrophy	Ref. [112]
Immunity and health	T cell reprogramming	Refs. [105,131]	Cardiovascular diseases	Ref. [152,153]
	Cellular stress response	Ref. [145]		
	Mitophagy	Refs. [114,121]		
	Apoptosis	Refs. [146]		
	Ageing	Refs. [120,124]		
Cell fate and development	Embryonic lethality	Refs. [16,17]	Stem cell differentiation	Ref. [154]
	Stem cell differentiation	Ref. [131,132]	Muscle homeostasis	Ref. [113]
	Oocyte development	Ref. [155]		
	Muscle homeostasis	Ref. [103]		
Metabolism	Cardiac health	Ref. [156]	Regulation of cellular metabolism	Ref. [68]
	Regulation of cellular metabolism	Ref. [104,105]	Obesity	Ref. [128]
	Obesity	Refs. [123,129,130]		
Cancer	Tumorigenesis	Ref. [134]		
	Brain-tumour-initiating cells	Ref. [135]		
	Breast cancer	Ref. [157]		

Effect of mutations in DRP1 and adaptor proteins on health and physiology.

disease (including encephalopathy, optic atrophy and neuromuscular defects)[112], and a mutation in MiD49 leads to muscle myopathy[113].

Quality control and organelle health

In healthy cells, mitochondrial fission is linked to quality control of the network and to changes influenced by metabolic state (Fig. 1). For example, the fission of mitochondria can act as a surveillance mechanism to identify poorly functioning daughter organelles that show reduced mitochondrial membrane potential[114]. These mitochondria may recover by fusing back with the network[115] or be targets of degradation through a form of macroautophagy known as mitophagy[116,117]. An attractive hypothesis suggests that smaller mitochondria generated from mitochondrial fission promote mitophagy, as these smaller organelles can be efficiently segregated from the mitochondrial population for degradation by the autophagic machinery[118]. Indeed, in cysts of female *Drosophila*, fragmentation of the mitochondrial network facilitates mitophagy and thus allows the selective clearance of defective mitochondria that contain mutant mtDNA from the germline[119]. Furthermore, the promotion of mitochondrial fission at mid-life by upregulating levels of Drp1 extends the lifespan of *Drosophila* through mitophagy of old organelles and rejuvenation of the remaining mitochondria[120]. DRP1 facilitates the fission and segregation of damaged regions of mitochondria, which allows for efficient engulfment and degradation of the mitochondrial fragment and spares the remainder of the organelle from degradation[121]. Whether the cytosolic-facing fission machinery selectively recognizes damaged regions within the organelle remains an open question.

Metabolic state

In 1915, Lewis and Lewis[1] noted that any one mitochondrion may change by fusion or division and that the variability in form may be 'connected with the metabolic activity of the cell'. Generally, cells with a more fragmented network are more glycolytic, whereas those with an interconnected network are more dependent on mitochondrial OXPHOS for energy. Drugs that block OXPHOS lead to fragmentation of the network[122]. This can be executed by the stress-activated protease OMA1 that generates S-OPA1 forms, which block fusion and enhance fission[88]. Metabolic changes can also alter Ca^{2+} homeostasis, which affects mitochondrial fission owing to the activation of calcineurin—a cytosolic phosphatase that activates DRP1 by dephosphorylation of Ser637[63]. By contrast, calcineurin deficiency leads to Ser637 hyperphosphorylation, reduced mitochondrial fission and increased mitochondrial respiration, and protects mice from obesity induced by a high-fat diet[123]. Phosphorylation at Ser637 has also been linked to circadian control of mitochondrial fission[4]. Inhibition of DRP1 and mitochondrial fission events blocks circadian oscillations in ATP produced from OXPHOS. Defects in this process may lead to increased cellular senescence[65,124].

In addition, AMPK activation has been found to trigger mitochondrial fission in cells treated with OXPHOS inhibitors. AMPK phosphorylates key serine residues on MFF that drive DRP1 recruitment and mitochondrial fission[68]. This may be performed along with phosphorylation of ARMC10, which is also executed by AMPK[125]. It is not clear whether AMPK-mediated activation of fission helps to drive cellular responses to energy stress through metabolic rewiring or mitophagy of damaged organelles[126]. Although the activation of mitochondrial fission may facilitate mitophagy and even cell death, it is also important for biogenesis. In fact, mTORC1—which stimulates anabolic processes, including mitochondrial biogenesis—has been identified as regulating mitochondrial fission by controlling translation of mitochondrial fission process 1 (MTFP1 (also known as MTP18)), a mitochondrial inner-membrane protein seen at levels that correlate with DRP1 phosphorylation status and activity[127]. Finally, recent work in mice has highlighted a link between MFF, mitochondrial fission and obesity[128]. During the development of obesity and insulin resistance, sphingolipids accumulate in nonadipose tissue and mitochondria become more fragmented. MFF directly interacts with $C_{16:0}$ sphingolipids derived from the ceramide synthase CERS6. Deletion of CERS6 leads to protection of mice from obesity: these mice show improved glucose metabolism, increased mitochondrial respiration and reduced mitochondrial fragmentation. Similarly, the presence of hepatocytes that lack MFF[128] or DRP1[129] leads to a block in obesity-associated mitochondrial fission and improves glucose metabolism.

Mitochondrial dynamics and the balance between fission and fusion are likewise important for metabolic reprogramming in specialized

cells. Proliferating T cells and cancer cells shift from oxidative phosphorylation to a glycolytic 'Warburg' state so that they maintain carbon units as building blocks for growth. For example, proliferating effector T cells are highly glycolytic and have largely fragmented mitochondria, whereas memory and regulatory T cells shift their metabolism towards OXPHOS and mitochondria are more elongated owing to decreased fission[130]. Metabolic reprogramming is also important during stem cell differentiation, during which a shift from oxidative to glycolytic metabolism occurs. DRP1 is phosphorylated during reprogramming, which leads to fragmentation of the organelles. This phosphorylation is dependent on activation of ERK1 and ERK2, and hints at an axis between mitochondrial morphology and stem cell fate[131]. Moreover, mitochondrial fission promotes cellular transformation mediated via oncogenic RAS. In this case, the pro-fission phosphorylation of DRP1 on Ser616 is stimulated by the MAPK–ERK1 and ERK2 pathway[132,133]. The loss of DRP1 or of Ser616 phosphorylation status inhibits RAS-induced transformation and tumour growth. It seems that DRP1 and mitochondrial fission are part of reprogramming, and support the glycolytic flux and other metabolic changes required for this process[134]. Knockdown or inhibition of DRP1 in brain-tumour-initiating cells resulted in a halt in tumour growth and higher levels of apoptosis, which suggests that modulation of DRP1 may represent a potential avenue for therapeutic treatment of these tumours[132,135].

Signalling and cell death

Calcium is a crucial cellular signalling molecule and its release is tightly controlled in both spatial and temporal space (Fig. 4). Mitochondria are excellent vessels for the regulation of signalling as both energy production and Ca^{2+} storage in the mitochondrial matrix are combined in one organelle, delivering energy and signalling molecules to the synapse[136,137]. Indeed, mitochondria are actively transported to synapses and may regulate synaptic activity[138–140]. Maintenance of small mitochondrial fragments suitable for axonal transport is performed by the fission machinery[141]. Mutants of DRP1 in flies and mice display impaired synaptic transmission and lower mitochondrial abundance in synapses[142,143]. After plasma membrane damage, Ca^{2+} influxes cause DRP1-mediated fragmentation, which allows for an increased Ca^{2+} load of organelles in close proximity to the site of damage—thereby triggering rapid cell polarization and a stress response[144]. DRP1 inactivation and changes in mitochondrial dynamics also impaired Ca^{2+} homeostasis, leading to muscle atrophy in mice[103] and defects in the ability of macrophages to clear phagocytosed apoptotic cells[145].

Although mitochondrial fission is required for numerous cell signalling events, wholesale DRP1-mediated mitochondrial fragmentation is a hallmark of BAX and BAK-mediated apoptosis[146]. Loss of DRP1 or adaptors[36,44] slows cristae remodelling events induced by BAX and BAK outer-membrane permeabilization, and this impairs cytochrome *c* release and downstream mtDNA efflux[147]. It is now known that activated BAX or BAK localizes to ER–mitochondrial constriction sites and triggers MAPL (also known as MUL1)-dependent DRP1 SUMOylation. SUMOylated DRP1 stabilizes mitochondria–ER contacts to facilitate Ca^{2+} signalling and cristae remodelling during apoptosis[148]. Transient blocking of mitochondrial fission may therefore have therapeutic advantages; for example, by protecting ischaemia-reperfusion injury of cardiomyocytes after myocardial infarction[149,150].

Outlook

Since the first observation of mitochondrial fission at the beginning of the twentieth century, our understanding of the process and the underlying molecular machinery has vastly expanded. We now appreciate that the mitochondrial divisome is embedded in an intricate regulatory network, which enables the modulation of mitochondrial dynamics by various stimuli. However, several questions remain unanswered, including why it is that mitochondrial fission in mammals is subject to such elaborate regulation. DRP1 undergoes several post-translational modifications that can inhibit or promote fission. Adaptors come in different flavours that act on DRP1 in different ways, and these adaptors too can be regulated by post-translational modifications. Finally, a complex and variable suite of organellar and cytoskeletal interactions influences mitochondrial fission, including deciding the actual scission site through membrane remodelling. The answer may lie in the need to dynamically manage mitochondrial networks while accommodating the physiological constraints imposed from diverse cellular shapes and functions in different tissues. Another important question regards the coordination of membrane remodelling processes that involve the outer and inner compartments, and the mechanisms by which they cross-talk. Without a sequential order of membrane scission, incorrect phospholipid membrane leaflets could mix—which would lead to mitochondria rupturing and cell death. The mitochondrial divisome, while relaying forces exerted from the cytosolic side of the outer membrane to the inner mitochondrial membrane, must therefore ensure that these compartments remain separate during fission. This may be achieved by the intrinsic ability of a constriction-based fission pathway to traverse a hemi-fission intermediate that ensures sequential and nonleaky fission of the two compartments. However, these speculations point to our lack of understanding of the fundamental cellular machinery that manages membrane remodelling and/or fission of the inner compartment. Although this Review has focused on fission mechanisms, mitochondrial fusion processes face similar hurdles, including complex lipid remodelling and regulation. The recent finding that mitochondrial fusion also occurs at the same ER–mitochondria contacts that mediate fission events[115] indicates underappreciated coregulation between these opposing processes to ensure a dynamic network[151].

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