Review

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Making connections: interorganelle contacts orchestrate mitochondrial behavior

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Mitochondria are highly dynamic organelles. During their life cycle they frequently fuse and divide, and damaged mitochondria are removed by autophagic degradation. These processes serve to maintain mitochondrial function and ensure optimal energy supply for the cell. It has recently become clear that this complex mitochondrial behavior is governed to a large extent by interactions with other organelles. In this review, we describe mitochondrial contacts with the endoplasmic reticulum (ER), plasma membrane, and peroxisomes. In particular, we highlight how mitochondrial fission, distribution, inheritance, and turnover are orchestrated by interorganellar contacts in yeast and metazoa. These interactions are pivotal for the integration of the dynamic mitochondrial network into the architecture of eukaryotic cells.

Dynamic mitochondria are engaged in multiple interactions with other organelles

About 1.5 billion years ago, an α-proteobacterium-like cell was engulfed and taken up by a phagocytic Archaea-type host cell. Remarkably, the prey managed to survive and multiply within its predator. It is unknown exactly when and how this happened, but this ancient feast probably was the most important trigger for the evolution of compartmentalized eukaryotic cells [1]. During its transformation from a once free-living bacterium into a contemporary mitochondrion, the endosymbiont lost its autonomy and established extensive genetic and functional relationships with the host cell. Most of its genes were lost or transferred to the host nucleus and elaborate mechanisms for organelle biogenesis and metabolite exchange evolved [2]. Although mitochondria are not connected to the vesicular transport system, the coevolution of the endosymbiont with the host cell's endomembrane system resulted in extensive interdependent relationships of mitochondria and other organelles.

Mitochondrial contacts with the ER have been known since the study of cellular ultrastructure by electron microscopy in the 1950s [3,4]. Similarly, direct contacts of mitochondria with the plasma membrane were revealed in neurons more than 50 years ago [5]. Advances in molecular biology and imaging technology allowed cell biologists to appreciate the dynamic nature of these interorganellar contacts and to understand their physiological functions. These contacts proved to be pivotal for the control of the complex behavior of mitochondria. In response to the cell's ever-changing physiological conditions, mitochondria constantly adapt their copy number, shape, and intracellular position via directed movements along cytoskeletal tracks and frequent fission and fusion. Moreover, damaged and surplus organelles separate from the mitochondrial network and are degraded by mitophagy, a selective form of autophagy [6–9]. Current research demonstrates that these processes largely depend on interactions with other organelles. Here we discuss recent advances to illustrate the contributions of the ER, plasma membrane, and peroxisomes to the control of mitochondrial behavior.

Mitochondrion–ER contacts

Contacts of mitochondria with the ER can be readily seen by light and electron microscopy in many cell types. It is

Glossary

Construct helping in mitochondrion–ER association (chiMERA): a chimeric protein that allows artificial mitochondrion–ER tethering. It comprises an N-terminal mitochondrial membrane anchor, GFP, and a C-terminal ER tail anchor.
Dynamin-related proteins (DRPs): large GTPases that mediate various membrane remodeling events, including membrane fission and fusion. The conserved mitochondrial fission DRP is termed Dnm1 in yeast and Drp1 in metazoa.
KillerRed: the chromophore of the KillerRed protein produces reactive oxygen species (ROS) on light irradiation. It can be used to induce local damage in cells.
Mitochondrion-associated membranes (MAMs): ER membranes that are physically associated with mitochondria. Established roles of MAMs include transport of phospholipids and calcium signaling.
Mitochondrion-derived vesicles (MDVs): MDVs bud off from mitochondria in mammalian cells and fuse with other organelles to deliver cargo. Known target organelles include peroxisomes and lysosomes.
Mitofusins: membrane-bound DRPs that mediate fusion of the mitochondrial outer membrane. Mammals have two isoforms, mitofusin 1 and mitofusin 2. Mitofusin 2 has a dual localization in mitochondria and ER. It functions in both mitochondrial fusion and mitochondrion–ER tethering.
Myosins: a large class of actin-dependent motor proteins. Most members of the myosin family, such as mammalian myosin II, have contractile properties, whereas others, such as class V myosins (including yeast Myo2), processively move along actin filaments to transport cargo.
Nucleoids: the mitochondrial genome is packaged into protein–DNA complexes called nucleoids. Budding yeast has about 10–40 nucleoids per cell, which are anchored to the mitochondrial inner membrane. Mammals typically have several hundred nucleoids per cell.
Omegasome: a cup-shaped membrane that is dynamically connected to the ER and serves as a platform for autophagosome biogenesis in mammalian cells.

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estimated that there are about 100 mitochondrion–ER contacts in a yeast cell [10] and about 5–20% of the mitochondrial surface is found in close proximity to the ER in HeLa cells [11]. Purification of mitochondrion-associated membranes (MAMs) (see Glossary) demonstrated that the ER and mitochondria are physically linked [12]. Mitochondrion–ER contacts play important roles in lipid transport and calcium signaling and several proteins involved in the establishment and function of these contacts have been identified (recently reviewed in [13–15]).

ER-associated mitochondrial division

The division of bacterial cells depends on FtsZ, a GTPase that self-assembles into a membrane-associated ring structure that coordinates the assembly of the division machinery [16]. Some primitive unicellular algae have retained FtsZ-related proteins from their bacterial ancestors to mediate mitochondrial fission. However, during the evolution of most eukaryotic lineages the prokaryotic division machinery was replaced by dynamin-related proteins (DRPs) [17,18]. In contrast to the bacterial division machinery, which acts on the inner side of the plasma membrane, the mitochondrial division machinery is assembled on the outside of the organelle. In yeast, the mitochondrial outer membrane protein fission 1 (Fis1) and the soluble adaptor mitochondrial division 1 (Mdv1) recruit dynamin 1 (Dnm1) to the mitochondrial surface. Metazoa possess two alternative dynamin-related protein 1 (Drp1) receptors, Fis1 and mitochondrial fission factor (Mff). Dnm1 and Drp1 recruited by these receptors self-assemble on mitochondria to form large, helical oligomers that wrap around the organelle and sever its membranes on GTP hydrolysis [6,8].

In vitro reconstituted Dnm1 spirals have a diameter of about 100 nm, which is too narrow to surround a 300-nm-thick mitochondrion [19]. Thus, constriction of the organelle must precede fission. Consistently it was shown in yeast that the mitochondrial diameter is reduced before Dnm1 assembles on the membrane [20]. For years it remained unknown how mitochondrial fission sites are selected and whether external forces contribute to mitochondrial constriction to allow assembly of the DRP division ring. An active role for the ER in defining the sites of mitochondrial fission proved to be the key to solving this problem. Electron tomography and live-cell fluorescence microscopy of yeast and mammalian cells revealed that the ER wraps around mitochondrial tubules before DRP recruitment. This activity marks the sites of fission and conceivably assists assembly of the DRP division ring by locally constricting the mitochondrial tubule to fit its diameter to that of the division machinery [21]. This process, which was termed ER-associated mitochondrial division [22], is conserved from yeast to mammals [21].

Intriguingly, mitochondrial constrictions at ER contacts were found even in the absence of Drp1 and Mff, suggesting that ER-dependent selection of fission sites occurs before assembly of the Drp1 division machinery [21]. The actin cytoskeleton contributes to this process, at least in mammalian cells. Inverted formin 2 (INF2), an ER-associated vertebrate formin that accelerates actin polymerization and depolymerization, promotes mitochondrial fission and actin filaments are present at the mitochondrion–ER interface [23]. In human osteosarcoma cells, myosin II accumulates at these sites in an INF2-dependent manner [24]. Based on these findings it was proposed that INF2-mediated actin polymerization and myosin II enable force generation to drive mitochondrial constriction and Drp1 assembly [23,24] (Figure 1A).

It is currently unknown whether the actin cytoskeleton is also required for ER-associated mitochondrial division in yeast. However, it has been shown that the actin-related protein 2/3 (Arp2/3) complex, a major initiator of actin polymerization, is associated with isolated yeast mitochondria and clouds of actin filaments can be seen around some mitochondria in intact yeast cells [25]. We consider it possible that these observations point to a role for actin polymerization in division of yeast mitochondria.

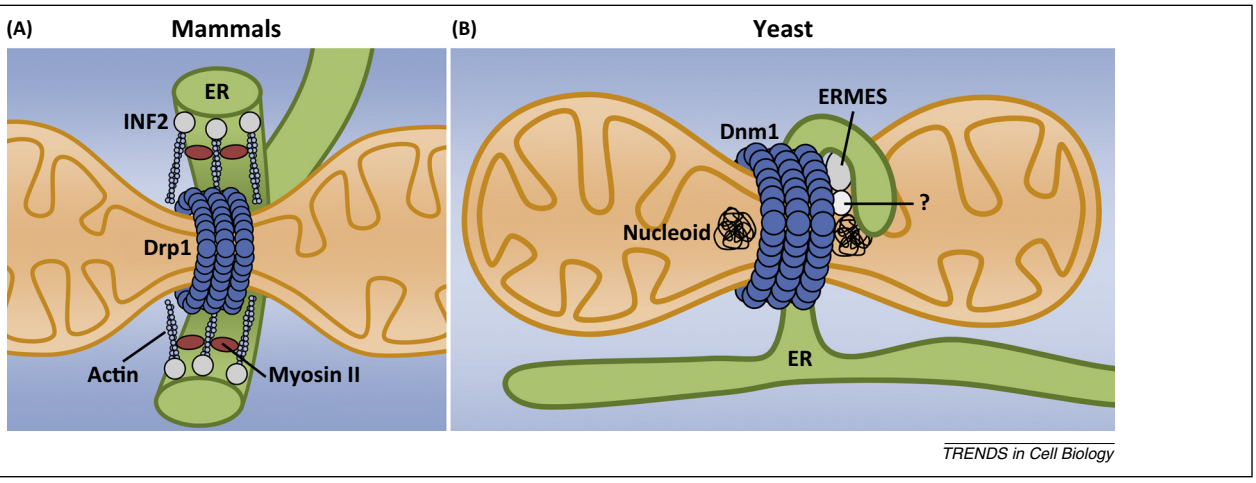

Figure 1. Endoplasmic reticulum (ER)-associated mitochondrial division. ER tubules wrap around mitochondria to mark sites of mitochondrial fission. This leads to mitochondrial constriction and supports assembly of rings of dynamin-related proteins (Drp1 in mammals and Dnm1 in yeast) that sever mitochondrial membranes on GTP hydrolysis [21]. **(A)** In mammals the ER-associated inverted formin 2 (INF2) is thought to contribute to ER-associated mitochondrial division by promoting actin filament polymerization at fission sites. The myosin motor myosin II could then generate forces to support assembly and activity of the Drp1 fission machinery [23,24]. **(B)** In yeast the ER is connected to mitochondria at fission sites by the ER–mitochondrion encounter structure (ERMES), a protein complex comprising subunits in the ER membrane and the mitochondrial outer membrane. ERMES is connected to nucleoids by yet unknown inner membrane proteins (?) and thereby aids in the partitioning of mitochondrial DNA to the two halves of the split organelle [22].

Box 1. The enigmatic role of the ERMES complex

The first screens for yeast mutants with aberrant mitochondrial morphology led to the identification of *MMM1*, *MDM10*, and *MDM12* in the 1990s [91–93]. About 10 years later, the *MDM34* gene (also known as *MMM2*) was described [66,94]. Since their discovery, different cellular functions have been proposed for these genes. Because mitochondria in mutant cells show defects in motility and inheritance, it was suggested that Mdm10, Mdm12, and Mmm1 function as a 'mitochore' that links mitochondrial membranes and DNA to actin cables to promote bud-directed movement [95]. Later, the same proteins were proposed to function in the biogenesis of β barrel proteins in the mitochondrial outer membrane [96,97]. More recently, ERMES was suggested to promote lipid transport between mitochondria and the ER [26]. However, although several studies found that mutant mitochondria have altered lipid composition [26,98–100], another study claimed that ERMES does not have a direct role in the transport of phosphatidylserine from the ER to mitochondria [101].

How can these conflicting observations and different models be reconciled? Although Mdm10 and Mdm34 are *bona fide* mitochondrial outer membrane proteins, some of the controversies in the literature can be ascribed to the fact that Mmm1 was erroneously localized to the mitochondrial outer membrane on its first characterization [91]. Not until 2009 was it found that the N terminus of Mmm1 is glycosylated, thereby unequivocally demonstrating its localization in the ER [26] (Figure I). It appears difficult to reconcile roles as a mitochore or biogenesis of mitochondrial β barrel proteins with the localization of Mmm1 in the ER. However, copurification experiments suggest a dual function in distinct complexes for Mdm10 [97]. A fraction is part of the sorting and assembly machinery (SAM) complex that functions in β barrel protein biogenesis, whereas another fraction is part of the ERMES complex. A role of ERMES in lipid transport is fully compatible with its localization at the mitochondrion–ER interface [14,26]. Consistently, Mmm1, Mdm12, and Mdm34 contain tubular lipid-binding (TULIP)-like domains that are known to bind lipids [102]. Apparently, mitochondrial defects in ERMES mutants can be readily compensated. Overexpression of Mdm10 complementing protein 1 (*MCP1*) or *MCP2*, two genes encoding mitochondrial proteins of poorly characterized function, restores mitochondrial morphology, distribution, and lipid composition in ERMES mutants [100]. GFP fusions of ERMES subunits revealed five to ten punctate structures per cell [26,29]. Even if this may be an underestimation [101], the available quantifications do not match the estimated 100 mitochondrion–ER contacts that can be seen by electron microscopy [10]. Thus, it appears that redundant pathways exist that could restore vital functions, such as lipid transport, in ERMES mutants and suppressor mutations readily accumulate. In our view, the available data are best compatible with a primary function of ERMES as a hub at the mitochondrion–ER interface coordinating mitochondrial fission, nucleoid partitioning, mitophagy (as discussed in the main text), and lipid transport [14]. Defects in mitochondrial morphology, motility, and protein assembly in mutants might be indirect consequences of compromised interactions with the ER and altered lipid composition of mitochondria.

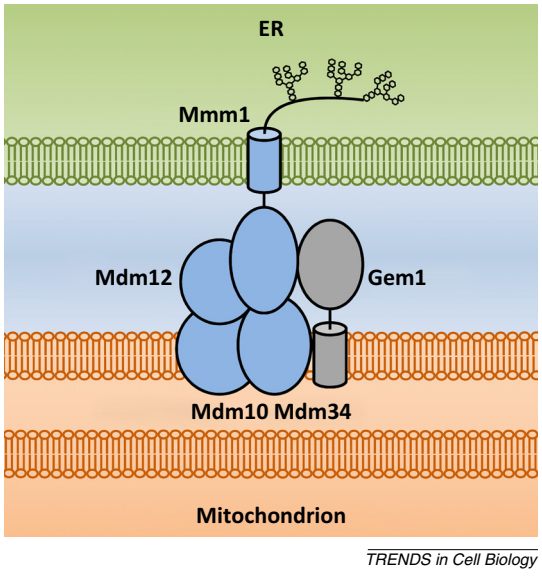

Figure I. The endoplasmic reticulum–mitochondrion encounter structure (ERMES).

In yeast, the ER–mitochondrion encounter structure (ERMES) forms mitochondrion–ER contacts. ERMES comprises subunits resident in both the ER and mitochondria: a glycosylated ER membrane protein, maintenance of mitochondrial morphology 1 (Mmm1); a soluble factor, mitochondrial distribution and morphology 12 (Mdm12); and two mitochondrial outer membrane proteins, Mdm10 and Mdm34 [26]. ERMES subunits have been implicated in various different cellular functions, some of which are controversial (Box 1). Deletion mutants lacking ERMES subunits have giant, swollen mitochondria and show growth deficits on nonfermentable carbon sources. A synthetic biology screen showed that these phenotypes can be rescued by expression of an artificial mitochondrion–ER tether, construct helping in mitochondrion–ER association (chiMERA) [26]. This observation assigns an important role to ERMES as a tether connecting mitochondria and the ER.

ERMES is present at sites of mitochondrial division and remains associated with only one of the two mitochondrial tips after fission [22]. GTPase EF-hand protein of mitochondria (Gem1), which is a regulatory subunit of ERMES [27,28], is involved in resolving ERMES-associated mitochondrial constrictions after fission [22]. Remarkably, ERMES subunits colocalize with actively replicating nucleoids [29,30]. These nucleoids often exhibit oscillatory movements and rapidly segregate and recoalesce before mitochondria divide. This results in partitioning of nucleoids and thereby facilitates mitochondrial DNA presence on both halves of the split organelle. Thus, it appears that ER-associated mitochondrial division links the distribution of mitochondria and nucleoids in yeast cells [22] (Figure 1B). Interestingly, mitochondrial fission often occurs adjacent to nucleoids in mammalian cells as well [31], suggesting that the cellular mechanisms responsible for spreading of nucleoids throughout the mitochondrial network might be conserved.

Although ERMES plays an important role in ER-associated mitochondrial division in yeast, functionally equivalent proteins in mammalian cells are currently unknown. ERMES is present in the fungal lineage and some protists, but not in metazoa [32]. Thus, higher eukaryotes must employ different machinery to establish mitochondrion–ER contacts. Mitofusin 2 is an obvious candidate, because it has been shown to tether mitochondria to the ER for calcium uptake [33]. However, mitofusin 2 is not required for ER-mediated mitochondrial constriction [21] and mitochondrion–ER contacts can form efficiently in cells lacking mitofusin 2 [34]. Mitochondrial rho-like GTPase (Miro-1) is

a mammalian homolog of yeast Gem1. It was suggested to function in the recruitment of kinesin motor proteins to mitochondria in axonal transport in neurons [35]. Interestingly, Miro-1 was recently found to localize to ER–mitochondrion contact sites in COS-7 cells [27]. However, a role of Miro-1 as part of a putative mammalian mitochondrion–ER tether remains to be shown and it is possible that the functions of Gem1 and Miro-1 are different in yeast and mammals.

ER-dependent mitochondrial distribution

Most membrane-bound organelles cannot be made *de novo* but must be inherited on cell division. In particular, asymmetrically dividing cells rely on cytoskeleton-dependent transport processes for partitioning of their organelles before cytokinesis [36]. Because mitochondria and ER are physically linked through numerous contact sites, it seems possible that their transport and inheritance is tightly coupled. However, studies in the budding yeast *Saccharomyces cerevisiae* suggest that independent inheritance mechanisms exist and that mitochondrion–ER contacts play a role in organelle partitioning, rather than transport.

During each cell cycle, yeast cells become polarized, select a site for bud emergence, and direct transport of secretory vesicles and organelles toward the growing bud. These transport processes are mediated by myosin motor proteins travelling along actin cables, which comprise bundles of actin filaments and provide tracks for directed organellar movements. When the bud approaches the size of the mother, a septum is formed, separating the cells and releasing the daughter from its mother. Bud-directed transport of mitochondria is mediated by Myo2 [37,38], a class V myosin, whereas cortical ER is transported by Myo4 [39], the second yeast class V myosin. Mutations of the Myo2 cargo-binding domain result in strong impairment of bud-directed mitochondrial transport, whereas inheritance of the ER remains normal [38]. Vice versa, deletion of the *MYO4* gene impairs transport of cortical ER to the bud, whereas inheritance of mitochondria remains normal [39]. These observations suggest that independent transport and inheritance systems exist for the two organelles.

Mitochondrial Myo2 receptor-related 1 (Mmr1) is an outer membrane protein that is present only on bud-localized mitochondria. It physically interacts with Myo2 and a role in recruitment of Myo2 to mitochondria has been discussed [40,41]. An alternative function was recently proposed based on the observation that Mmr1 is seen at mitochondrion–ER contact sites in the bud by super-resolution microscopy. It was suggested that Mmr1 establishes direct contacts between mitochondria and cortical ER in the bud, thereby creating an anchor to prevent backward movement of newly inherited mitochondria and ensure efficient inheritance of mitochondria [42]. The observations that deletion mutants lacking Mmr1 accumulate mitochondria in the mother cell and show decreased mitochondrial content in the bud [40,42–44] are consistent with both proposed functions. Thus, more work is required to define the role of Mmr1 in mitochondrial inheritance.

Cooperation of ER and mitochondria in autophagy

Autophagy is a process responsible for the turnover of long-lived proteins and degradation of damaged or excess organelles. Cargo destined for degradation is engulfed by a double membrane called the phagophore or isolation membrane. Its edges seal to form the mature autophagosome. The autophagosome then fuses with the lysosome or vacuole to deliver its contents for degradation [45]. Over 30 autophagy-related (Atg) proteins are known to participate in autophagic pathways. Many components of this machinery are conserved from yeast to mammals [46]. During engulfment of its cargo, the phagophore membrane has to grow considerably before it becomes a mature autophagosome. For decades, it has been a matter of debate how the phagophore membrane grows and where the membrane material comes from [46,47]. Several recent studies indicate an important role of mitochondrion–ER contacts in this process.

Cells induce autophagy on starvation to retrieve nutrients from degradation of their own components. In starved mammalian cells, it was shown that autophagosomes are formed in the vicinity of mitochondria [48,49] and that mitochondria transiently share membranes with autophagosomes to supply them with phospholipids [48]. Intriguingly, formation of autophagosomes is impaired by depletion of mitofusin 2 [48,49], a known tether connecting mitochondria and the ER [33], or phosphofurin acidic cluster sorting protein-2 (PACS-2) [49], a cytosolic protein implicated in ER–mitochondrion communication [50]. It was proposed that the phospholipid phosphatidylserine is transported from the ER to mitochondria, where it is converted to phosphatidylethanolamine and can be used for autophagosome biogenesis [48]. Consistent with this model, two autophagosomal marker proteins assemble at mitochondrion–ER contact sites [49]. Atg14, a subunit of an autophagy-specific phosphatidylinositol 3-kinase complex [51], is recruited by the ER-resident SNARE syntaxin 17 [49] and Atg5, a marker of immature phagophores [52], remains at mitochondrion–ER contacts until autophagosome formation is complete [49]. These observations assign an important role to mitochondrion–ER contacts in general autophagy. Presumably, both organelles cooperate to supply the growing phagophore membrane with lipids (Figure 2A).

Mitophagy is a selective form of autophagy that specifically eliminates mitochondria, which is important during cell developmental processes, such as maturation of erythrocytes, or for mitochondrial quality control in situations where the accumulation of dysfunctional mitochondria becomes harmful to the cell [9]. In mitophagy, mitochondria are substrates of autophagy rather than active players. This process occurs at the mitochondrion–ER interface too. In mammalian cells, damaged mitochondria were observed to be engulfed by autophagic structures at specialized ER regions [53] termed omegasomes [54]. Similarly, it was found in yeast that mitophagy is induced at mitochondrion–ER contact sites marked by ERMES components Mdm12 and Mdm34. Initially, it was suggested that ERMES is present at these sites to promote ER-associated mitochondrial division, which is necessary to generate mitochondria that are small enough to be

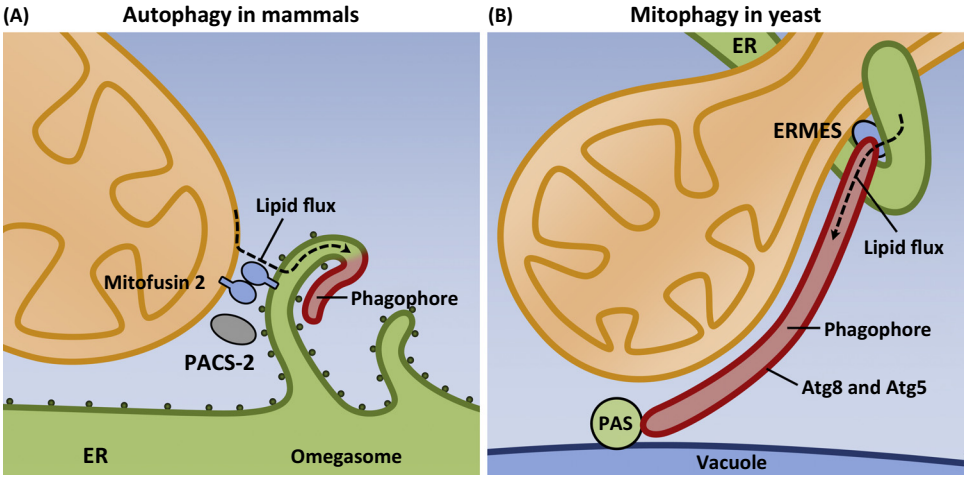

Figure 2. Role of mitochondrion–endoplasmic reticulum (ER) contacts in autophagy. Two models suggest that growth of the phagophore occurs at the mitochondrion–ER interface. **(A)** In general autophagy, mammalian mitofusin 2 and phosphofurin acidic cluster sorting protein-2 (PACS-2) are involved in connecting mitochondria to the ER to allow flux of membrane lipids to the phagophore at a site termed the omegasome [48,49]. **(B)** Tethering of mitochondria to the ER by the ER–mitochondrion encounter structure (ERMES) complex is crucial for mitophagy in yeast. It was proposed that this activity might be important in facilitating lipid flux from the ER to the phagophore to support growth of the phagophore membrane [56]. The phagophore contains the autophagy-related (Atg) proteins Atg5 and Atg8 and is initiated at the phagophore assembly site (PAS), which is located in the vicinity of the vacuole.

engulfed by the mitophagophore [55]. However, more recently it was shown that ERMES plays a direct role in yeast mitophagosome biogenesis. ERMES mutants have a severe mitophagy defect and accumulate immature mitophagosomes marked by Atg5. These defects can be rescued by restoration of mitochondrion–ER contacts by chiMERA expression, demonstrating that mitochondrion–ER contacts *per se* are important. Furthermore, Mmm1 was found to interact with Atg8, a phagophore membrane marker. It was suggested that ERMES tethers the mitochondrion destined for degradation to the ER and the growing phagophore. This activity is thought to ensure an efficient supply of lipids from the ER to promote growth of the phagophore, which has to engulf a rather large cargo during mitophagy [56] (Figure 2B). Interestingly, other autophagic pathways appear not to be compromised in ERMES mutants, suggesting that the role of mitochondrion–ER contacts is specific for mitophagy in yeast.

The mitochondrion–plasma membrane contact site

Mitochondria are positioned close to the plasma membrane in several mammalian cell types. In HeLa cells, for example, about 10% of the plasma membrane is covered with mitochondria [57]. Interestingly, on Fis1 or Drp1 overexpression-induced fragmentation most mitochondria redistribute away from the plasma membrane [57], suggesting that mitochondria are connected to the cell cortex only at a few anchor points. Consistently, experiments with drug-inducible fluorescent interorganelle linkers reveal that most mitochondria are not in direct contact with the plasma membrane in rat leukemia cells and cardiomyocytes. Rather, mitochondria appear to be connected to the plasma membrane through an ER stack, a connection important for calcium signaling [58]. By contrast, certain neurons with high rates of vesicle membrane turnover contain a specialized organelle assembly comprising an adherence plaque, which is associated by filamentous strands with a mitochondrion [59]. These mitochondria have a unique ultrastructure, but the molecules building the mitochondrial plaque and its physiological role remain elusive [60]. A recent proteomic study suggests that the plasma membrane connexin Cx32, a structural subunit of gap junctions, interacts with mitochondrial proteins in murine hepatocytes. It was proposed that this activity transiently tethers mitochondria to Cx32-enriched plasma membrane microdomains [61]. However, most of the molecular components and physiological functions of mitochondrial plasma membrane contacts in metazoa remain unknown.

Recently, the first molecular and functional characterization of a mitochondrion–plasma membrane contact site was reported in yeast [43,62]. Nuclear migration 1 (Num1) is a large, 313-kDa protein that binds to the plasma membrane via its C-terminal pleckstrin homology (PH) domain and assembles into cortical patches [63,64]. In mitotically growing cells these patches are found mainly in the mother cell and appear in the bud only late in the cell division cycle [65]. One major function of Num1 is to provide a cortical attachment site for dynein-dependent sliding of microtubules during positioning of the mitotic spindle [65]. Unexpectedly, deletion of the *NUM1* gene was found also to cause severe defects in mitochondrial morphology and positioning [66,67]. Mitochondria are normally located directly below the cell cortex in wild type yeast cells. This peripheral localization is lost in Δ*num1* mutants and mitochondria move more freely and faster than wild type mitochondria, suggesting that restraints impeding mitochondrial motility are lacking [62,64,67,68]. Num1 patches at the cell cortex colocalize with mitochondria and conceivably represent cortical anchoring sites [43,62,67]. Importantly, mitochondrial distribution and morphology defects in Δ*num1* mutants can be rescued by expression of synthetic plasma membrane tethers [43,62], demonstrating that attachment to the plasma membrane is important. These observations suggest that

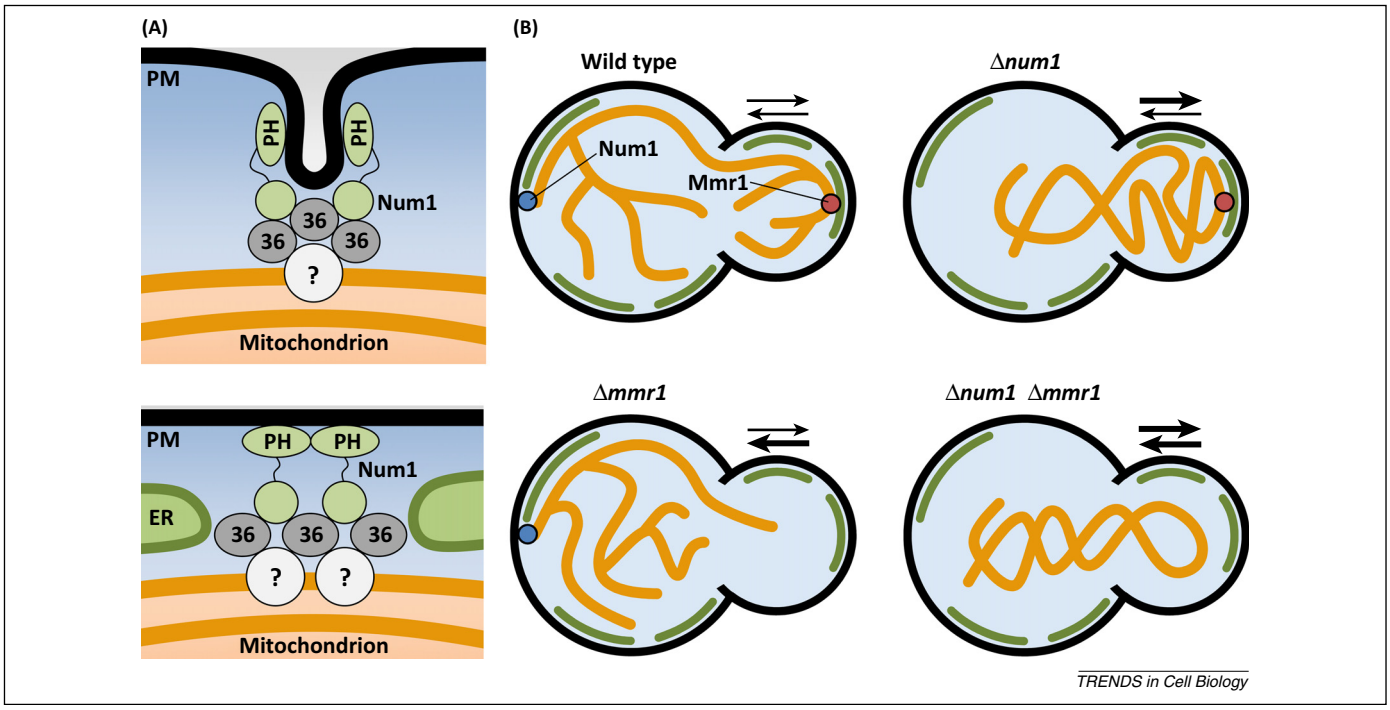

Figure 3. Role of cortex anchors in mitochondrial partitioning in budding yeast. **(A)** Two models for mitochondrial plasma membrane (PM) attachment sites in the mother cell. Nuclear migration 1 (Num1) binds to the PM via its pleckstrin homology (PH) domain and forms cortical patches in the mother cell. The peripheral mitochondrial adapter protein mitochondrial distribution and morphology 36 (Mdm36) (36) is thought to connect these patches to a yet unknown receptor (?) in the mitochondrial outer membrane. Electron tomography of mitochondrial PM anchors revealed direct contacts of the mitochondrial outer membrane with invaginations of the PM (top) [43], whereas light microscopy and proteomic analyses suggest participation of the endoplasmic reticulum (ER) to form mitochondrion–ER–cortex anchors (bottom) [62]. **(B)** Role of Num1 and mitochondrial Myo2 receptor-related 1 (Mmr1) cortex anchors in mitochondrial partitioning. In wild type cells, equal distribution of the mitochondrial network (orange) depends on balanced anterograde and retrograde movements (arrows). Furthermore, faithful partitioning is thought to be ensured by Num1 cortex anchors connecting mitochondria to the PM in the mother and Mmr1 connecting mitochondria to cortical ER (green) at the bud tip. In Δ*num1* mutants mitochondrial distribution is shifted toward the bud because retention sites in the mother are lacking. In Δ*mmr1* mutants mitochondrial distribution is shifted toward the mother because retention sites in the bud are lacking. In Δ*num1* Δ*mmr1* double mutants, equal distribution of mitochondria is restored because mitochondria can move freely in both directions without the constraints of cortex anchors [42,43,62].

Num1 is a component of a cell cortex anchor tethering mitochondria to the plasma membrane in the mother cell.

Proteomic and yeast two-hybrid analysis of Num1 identified Mdm36 as the second component of the tethering complex [62]. Mdm36 peripherally associates with mitochondria [68] and is located in foci adjacent to Num1 punctae [62], and Δ*mdm36* mutants resemble Δ*num1* mutants with respect to their mitochondrial phenotypes [68]. Thus, Mdm36 appears to be a mitochondrion-specific adaptor protein connecting the organelle to a multifunctional plasma membrane attachment site provided by Num1. Because contacts of Num1 with mitochondria can be observed in the absence of Mdm36 [62], additional components are likely to be involved. Although one study proposed that the ER actively contributes to this process [62], electron tomography revealed direct contacts of mitochondria and invaginations of the plasma membrane without participation of the ER [43] (Figure 3A). Thus, more work is needed to understand the molecular nature of mitochondrion–plasma membrane contacts.

Interestingly, the Δ*num1* deletion shows positive genetic interactions with the Δ*mmr1* deletion [69], suggesting that both genes have antagonistic functions. Whereas Mmr1 was proposed to anchor mitochondria to cortical ER in the bud (see above), Num1 apparently plays a similar role in the mother cell. Impairment of mitochondrial dynamics or enforcement of anterograde mitochondrial transport in Δ*num1* cells leads to the formation of mother cells that are depleted of mitochondria [43,62,67], suggesting that Num1 is required as a mitochondrial retention factor in the mother cell. Thus, mitochondria appear to be attached to the mother cell cortex by Num1 and to the bud tip by Mmr1 and the ER. According to this model, the joint activity of Num1 and Mmr1 ensures faithful partitioning of mitochondria to the mother cell and the bud on cell division (Figure 3B).

Metazoan homologs of Mdm36, Mmr1, or Num1 are unknown. Probably, most mammalian cells do not strictly depend on precisely controlled partitioning mechanisms. Instead, mitochondria are thought to be distributed in a stochastic manner during cell division [36,70]. Nevertheless, highly differentiated metazoan cell types also rely heavily on a balance of mitochondrial trafficking and immobilization. In neurons, for example, downregulation of motor activity and tethering to cytoskeletal elements contribute to mitochondrial immobilization at sites of high energy demand [71–73]. It appears that different systems have evolved that govern mitochondrial partitioning and distribution in eukaryotic cells.

The peroxisome–mitochondrion connection

Despite their different evolutionary origins, mitochondria and peroxisomes are engaged in a surprisingly close interrelationship, the 'peroxisome–mitochondrion connection' [74]. The two organelles act in common metabolic pathways, including β-oxidation of fatty acids and scavenging of peroxides. Strikingly, peroxisomes and mitochondria share key components of their division machinery, including

Drp1 and its receptors Fis1 and Mff in mammals and Dnm1 and Fis1 in yeast [75,76]. Several lines of evidence suggest that physical connections between mitochondria and peroxisomes exist, at least in some cell types. Mitochondria can be copurified with peroxisomes from rat liver cells [77], directed peroxisomal movements occur in association with mitochondria in fission yeast [78], and peroxisomes were found to closely associate with the ER and mitochondria in electron micrographs of budding yeast cells [79].

Although it is unknown whether physical contacts allow the exchange of metabolites between mitochondria and peroxisomes, mitochondrion-derived vesicles (MDVs) were suggested to deliver contents to peroxisomes in mammalian cells [80]. The physiological function of this process is not completely understood, but it may contribute to peroxisomal biogenesis [81]. Although MDVs are thought to constitute unidirectional transport vehicles from mitochondria to peroxisomes, circumstantial evidence suggests that peroxisomes may also influence mitochondrial behavior. It was observed that induction of oxidative stress by expression of peroxisome-targeted KillerRed induces excessive mitochondrial fragmentation in mammalian cells [82]. Although the functional significance of this phenomenon is currently unclear, it might reflect a common role of mitochondria and peroxisomes in redox-sensitive signaling processes. Recent work in yeast reported that degradation of peroxisomes by pexophagy, a peroxisome-specific form of autophagy, depends on peroxisomal division [83]. Intriguingly, the scaffold protein Atg11 recruits the Dnm1 fission machinery to the organelle destined for degradation in both mitophagy [55] and pexophagy [83] and pexophagy-specific division is initiated at mitochondrion–peroxisome contact sites [83]. These observations indicate intense crosstalk of organelles during autophagy.

Concluding remarks

Cell organelles were long regarded as secluded compartments tailored for the specific needs of distinct cellular pathways. It is now becoming clear that organelles are engaged in networks of cooperative activities. Importantly, interorganellar connections must be dynamic to allow exchange of metabolites, coordination of signaling activities, membrane fission, or degradation by autophagy to adapt organellar functions to the physiological conditions of the cell. Tight coordination of these activities is essential to establish and maintain the intricate architecture of eukaryotic cells. Thus, the investigation of organelle contacts sites and their molecular components is instrumental for our understanding of the eukaryotic cell.

Although mitochondrion–ER contacts and their functions in calcium signaling and lipid transport have been studied for a long time [14,15], the identification of the role of the ER in mitochondrial division, nucleoid partitioning, and mitophagy is rather recent. Several important questions remain. For example, the molecular tethers of ER-associated mitochondrial division are still unknown in mammalian cells and it is unclear whether mitochondrion–ER contacts are of general importance in autophagy in yeast. A recent study suggests a link between mitochondrion–ER contacts, mitochondrial dynamics, and Parkinson's disease [84] (Box 2). Thus, further studies of these processes are likely to contribute to our understanding of disease mechanisms.

Accumulating evidence also revealed that contacts with the plasma membrane and peroxisomes contribute to mitochondrial dynamics and inheritance, demonstrating that mitochondrial function is determined by interactions with multiple cellular membranes. We consider it likely that additional, but poorly characterized, contacts with other organelles exist. For example, dysfunctions of the lysosome-like vacuole in yeast compromise mitochondrial function. Deletion of genes required for vacuolar biogenesis and function frequently leads to loss of respiratory functions [85,86] and age-associated decline of vacuolar acidity limits mitochondrial function and lifespan [87]. In mammals, lysosomes are connected to mitochondria by MDVs in a process that contributes to mitochondrial quality control [88,89]. Recently it was shown that melanosomes, which are specialized lysosome-related organelles in mammalian pigment cells, establish physical contacts with mitochondria in a mitofusin 2-dependent manner. The functional significance of these contacts is not yet clear, but they might contribute to melanosome biogenesis through exchange of calcium, control of the redox status in melanocytes, or supply of ATP to melanosomes [90]. Thus, the network of interorganellar interactions that determines mitochondrial function and behavior might prove to be even more complex and its investigation will remain an exciting field of research in the coming years.

Box 2. A possible role of mitochondrion–ER contacts in Parkinson's disease

Parkinson's disease is a neurodegenerative disorder characterized by the loss of dopaminergic neurons. Neuronal cell loss is caused by the accumulation of dysfunctional mitochondria and a concomitant increase of ROS [103]. Mutations of PTEN-induced putative kinase protein 1 (PINK1) and parkin are associated with Parkinson's disease. In healthy cells, PINK1 accumulates on the surface of damaged mitochondria and recruits the ubiquitin ligase parkin to mitochondria. Parkin then ubiquitinates mitochondrial substrates and thereby initiates mitophagy to remove dysfunctional mitochondria from the cell. Defects in this quality control system lead to the accumulation of malfunctioning mitochondria with detrimental consequences for the cell [7,9]. Known substrates of parkin include proteins that are present at the mitochondrion–ER interface, such as mitofusin 2 [104] and Miro-1 [105]. Consistently, mitophagosomes form at mitochondrion–ER contact sites [53]. It was shown that elevated parkin levels increase ER–mitochondrion tethering and calcium crosstalk, whereas knock down of parkin leads to a reduction of mitochondrion–ER contacts, indicating a direct role of parkin in regulation of these contacts [106]. Another protein implicated in Parkinson's disease is α-synuclein, which forms cytoplasmic inclusion bodies, termed Lewy bodies, that are found in affected neurons [107]. Intriguingly, wild type α-synuclein is enriched in mitochondrion-associated ER membranes in several cell lines and tissues. Pathogenic mutations of α-synuclein result in reduced localization to mitochondrion–ER contact sites, decreased mitochondrion–ER tethering, and increased mitochondrial fragmentation [84]. Taken together, these data suggest that mitochondrion–ER contacts have important functions in mitochondrial homeostasis and that their disturbance might result in severe damage in vulnerable cells such as neurons. These observations might have significant implications for our understanding of Parkinson's disease.

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