Review

CellPress

Making connections: interorganelle contacts orchestrate mitochondrial behavior

Till Klecker*, Stefan Böckler*, and Benedikt Westermann

Institut für Zellbiologie, Universität Bayreuth, 95440 Bayreuth, Germany

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.

Corresponding author: Westermann, B. (benedikt.westermann@uni-bayreuth.de).
Keywords: endoplasmic reticulum; mitochondria; organelle contact sites; peroxisomes; plasma membrane.
*These authors contributed equally to this work.

0962-8924/
 http://dx.doi.org/10.1016/j.tcb.2014.04.004

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.

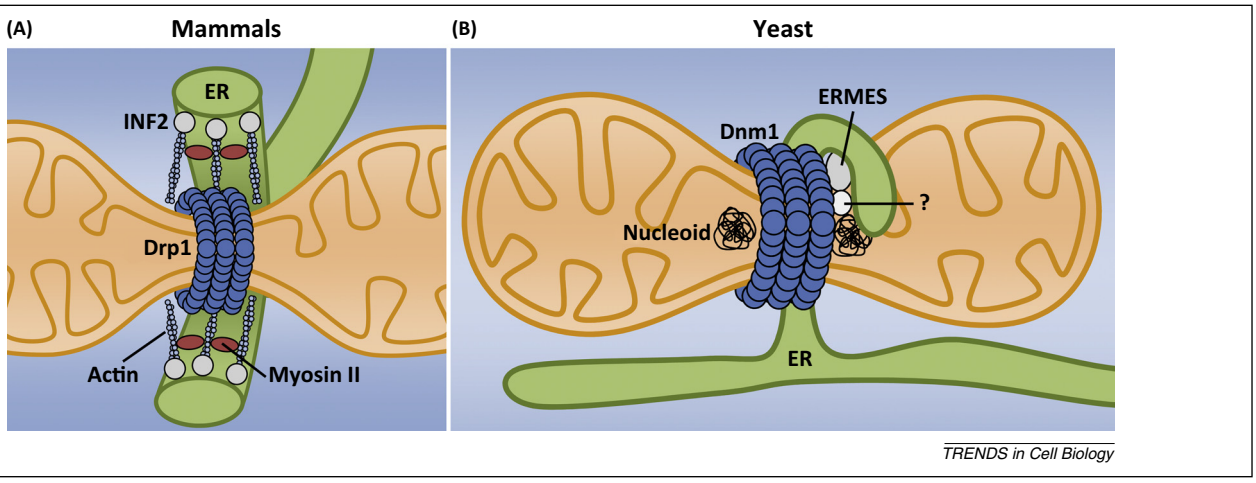

Figure 1. Endoplasmic reticulum (ER)-associated mitochondrial division. ER tubules wrap around mitochondria to mark sites of mitochondrial fission. This leads to mitochondrial constriction and supports assembly of rings of dynamin-related proteins (Drp1 in mammals and Dnm1 in yeast) that sever mitochondrial 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].