# Determinants and Functions of Mitochondrial Behavior

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## Abstract

Mitochondria are ancient organelles evolved from bacteria. Over the course of evolution, the behavior of mitochondria inside eukaryotic cells has changed dramatically, and the corresponding machineries that control it are in most cases new inventions. The evolution of mitochondrial behavior reflects the necessity to create a dynamic compartment to integrate the myriad mitochondrial functions with the status of other endomembrane compartments, such as the endoplasmic reticulum, and with signaling pathways that monitor cellular homeostasis and respond to stress. Here we review what has been discovered about the molecular machineries that work together to control the collective behavior of mitochondria in cells, as well as their physiological roles in healthy and disease states.

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## INTRODUCTION

Mitochondria are double membrane endosymbiotic organelles that are at the heart of eukaryotic cell metabolism (Nunnari & Suomalainen 2012). Their hallmark ability to efficiently catalyze the production of ATP via oxidative phosphorylation made them pivotal players in the evolution of the eukaryotic cell (Lane & Martin 2010). Their transformation into efficient energy machines was enabled by a reduction in the size of the symbiont genome and the corresponding transfer of many of the genes to the nucleus (Gabaldon & Huynen 2007). As a consequence, the human mitochondrial chromosome is only 16 kb in size but still encodes essential RNA components of the mitochondrial translation system and 13 proteins, which are core constituents of the mitochondrial respiratory complexes I–IV embedded in a specialized invaginated region of the inner membrane, termed crista, where they form supermolecular complexes that work cooperatively.

Respiratory complex subunits encoded by the mitochondrial and nuclear genomes coordinately combine in a balanced manner that is tightly regulated and monitored by stress signaling pathways, such as the mitochondrial unfolded response (Pellegrino et al. 2013). Respiratory complexes catalyze the transfer of electrons from NADH generated by the TCA cycle to create a proton motive force across the inner membrane, used to drive ATP production via the turbine action of the ancient terminal ATP synthase complex. The mitochondrial electrochemical gradient is an essential feature of the organelle, driving many fundamental processes, such as organelle biogenesis via protein import and calcium buffering. Loss of the electrochemical gradient is emerging as a signal for mitochondrial dysfunction to activate stress pathways.

Most of the mitochondrial proteome is nuclear-encoded and composed of a mosaic of proteins derived not only from the endosymbiont but also from other prokaryotic and eukaryotic origins (Flis & Daum 2013, Forner et al. 2006, Mootha et al. 2003, Sickmann et al. 2003). As a consequence, extant mitochondria have acquired biogenesis machinery to import nuclear-encoded proteins and lipids (Neupert & Herrmann 2007, Schmidt et al. 2010). They have also acquired new machinery that is responsible for controlling their behavior inside the cell. This machinery generates a dynamic mitochondrial compartment, whose structure and function are highly integrated with cellular status and with another ancient endomembrane, the endoplasmic reticulum (ER), which, as we review, is emerging as an intimate partner (also reviewed in Rowland & Voeltz 2012).

The dynamic nature of mitochondrial behavior manifests in the array of structures mitochondria form at steady state in different human cell types, ranging from highly connected tubular structures distributed throughout the cell, as observed in cardiomyocytes, to fragmented aggregates localized to a defined cellular position, as in oocytes (Kasahara et al. 2013, Pepling et al. 2007). The heteromorphic and dynamic nature of mitochondria is required for the placement of these organelles at sites of energetic demand and is the product of diverse machineries that control fusion, division, positioning, motility, and ultrastructural organization. Mitochondrial behaviors must also coordinate the distribution and accurate inheritance of mtDNA to maintain mitochondrial function. The mitochondrial chromosome is packaged into proteinaceous complexes termed nucleoids, which are present in cells at copy numbers that are in vast excess to that of nuclear chromosomes and are distributed throughout mitochondrial networks (as reviewed in Bogenhagen 2012). Here, we provide an overview of the molecular features and mechanisms of the machines that control mitochondrial behavior, mtDNA transmission, lipid composition, and internal architecture and describe how the events they catalyze are harnessed for mitochondrial and physiological functions in homeostasis and stress conditions.

## DYNAMIN-RELATED GTPASES CONTROL MITOCHONDRIAL DYNAMICS

The opposing processes of division and fusion work in concert to maintain the appropriate shape, size, and number of mitochondria (Bleazard et al. 1999, Nunnari et al. 1997, Sesaki & Jensen 1999). Reduced mitochondrial fusion results in uncountered division and fragmented mitochondria, whereas impaired division leads to a hyperfused mitochondrial network (Hoppins et al. 2007). This morphological remodeling is critical for regulating organelle physiology and acts along with motility and tethering mechanisms to adapt mitochondrial positioning and function to the bioenergetics needs of the cell. Remarkably, the machines responsible for mitochondrial division and fusion belong to the same family of highly conserved large dynamin-related GTPase proteins (DRPs) that, through their ability to self-assemble and hydrolyze GTP, control membrane remodeling events (Danino & Hinshaw 2001, Hoppins et al. 2007, Praefcke & McMahon 2004, van der Bliek 1999). This is in contrast to their bacterial ancestors, which use the cell cycle–dependent placement of a tubulin-like FtsZ cell-division machine to coordinate cell division with chromosome segregation. Except in the most basal eukaryotes, mitochondria have lost FtsZ-like and also actin-like cytoskeletal proteins (Erickson 2000, Osteryoung & Nunnari 2003). However, as we discuss later, mitochondrial fusion and division activities are still critical for the distribution and inheritance of mtDNA in organisms ranging from yeast to humans.

Dynamin GTPases are found in all kingdoms of life, and evidence suggests that their shared function is membrane remodeling. The prototypic member of the DRP family, dynamin, functions by forming helical structures that wrap around the necks of clathrin-coated pits to mediate their scission from the plasma membrane during endocytosis (as reviewed in Faelber et al. 2013, Hinshaw 2000, Schmid & Frolov 2011). Similarly, mitochondrial division is catalyzed by a cytosolic DRP, DRP1/Dnm1, in mammals/yeast that forms helical structures whose dimensions match mitochondria (Ingerman et al. 2005, Labrousse et al. 1999, Yoon et al. 2001). Fusion of the mitochondrial outer and inner membranes requires the action of two evolutionarily distinct integral membrane DRPs, MFN1 and MFN2/Fzo1 in mammals and yeast and OPA1/Mgm1 in mammals and yeast, respectively (Chen et al. 2003, Faelber et al. 2011, Hales & Fuller 1997, Hermann et al. 1998, Meeusen et al. 2006, Santel & Fuller 2001). The importance of balanced mitochondrial dynamics is exemplified by the severe pathophysiological consequences of disrupting any of these processes. Mice genetically deficient in MFN1, MFN2 (Chen et al. 2003), OPA1 (Alavi et al. 2007,

Davies et al. 2007), or DRP1 (Ishihara et al. 2009, Wakabayashi et al. 2009) do not survive past mid-gestation, and pathogenic mutations in the human orthologs of MFN2 and OPA1 lead to the neurodegenerative diseases Charcot-Marie-Tooth syndrome (CMT) and dominant optic atrophy, respectively (Alexander et al. 2000, Delettre et al. 2000, Waterham et al. 2007, Zuchner et al. 2004).

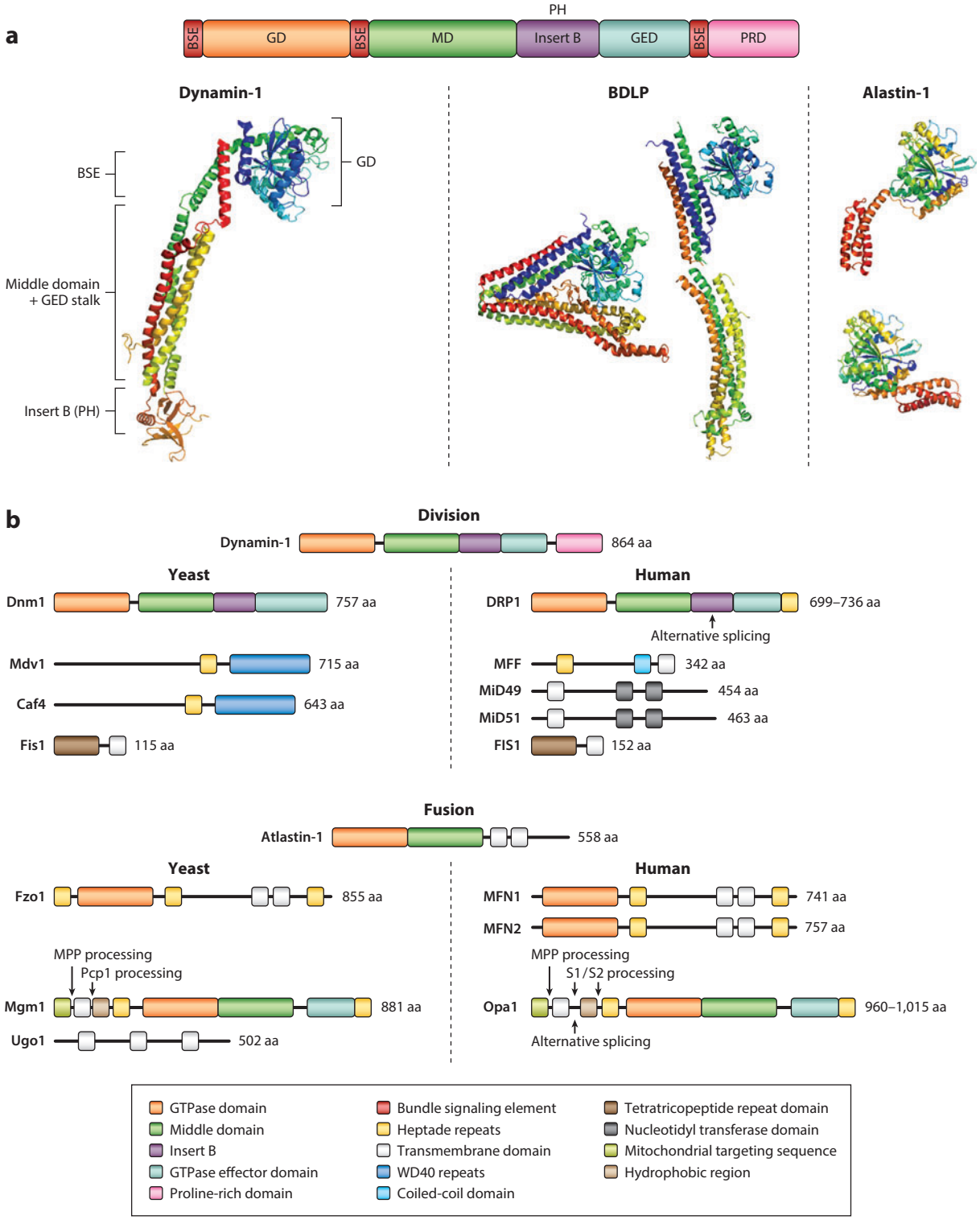
Phylogenetic analysis suggests that the outer mitochondrial membrane fusion DRP MFN/Fzo1 is related to bacterial DRPs, evolved from an ancestral progenitor (Bramkamp 2012). Although their physiological roles are poorly described, most bacteria encode two DRPs from a gene duplication event, organized in an operon, or, in some cases, such as in *Staphylococcus aureus*, two DRP gene paralogs encode a single tandem DRP protein (Burmann et al. 2011). Evidence suggests that bacterial DRP paralogs coassemble, with each performing a nonredundant function (Burmann et al. 2011). This theme is also apparent in the mitochondrial fusion dynamins, where, in metazoans, outer membrane fusion is mediated by the two distinct, but highly related, MFN1 and MFN2 proteins, which can form heterocomplexes but are functionally nonredundant (Detmer & Chan 2007). The inner membrane fusion DRPs, Mgm1 and OPA1, are processed by proteases to produce both long, membrane-associated and short, soluble isoforms. They also perform nonredundant roles in fusion and potentially function independently in the regulation of additional membrane shaping pathways, such as mitochondrial division and inner membrane crista formation, in an isoform-specific manner (Frezza et al. 2006, Griparic et al. 2007, Herlan et al. 2003, McQuibban et al. 2003, Meeusen et al. 2006, Song et al. 2007).

Biochemical and structural analyses have illuminated shared mechanisms of action of DRPs. DRPs possess up to four identifiable regions: a highly conserved GTPase (G) domain; two helical regions, which have been termed the middle domain (MD) and GTPase effector domain (GED); and a highly variable region between the MD and GED, termed insert B (**Figure 1**). These elements function together to mediate the self-assembly of DRPs into variable structures, which associate with membranes and influence their structure. Self-assembly requires GTP, and formation of these higher-order structures stimulates GTP hydrolysis, which is essential for DRP function (Ingerman et al. 2005, Muhlberg et al. 1997, Warnock et al. 1996). In vitro studies have demonstrated that DRPs typically have relatively low affinities for GTP and GDP (Eccleston et al. 2002), but the rates of assembly-stimulated GTP hydrolysis vary greatly among the members. Higher rates have been reported for members mediating membrane scission in comparison to membrane fusion members, which may be relevant for mechanistic differences in these opposing processes.

Atomic structures of diverse members of the DRP family from bacteria to human indicate that the MD and a portion of the GED cooperate to form a helical bundle, termed the stalk (Faelber et al. 2011, Ford et al. 2011, Frohlich et al. 2013, Gao et al. 2010, Low et al. 2009, Prakash et al. 2000). The stalk domains of DRPs encode information for the specificity of DRP oligomerization and for the geometry of higher-order DRP assemblies. In the scission DRPs, such as dynamin and

**Figure 1**

Common architecture of dynamin-related proteins. (*a*) (*top*) Schematic representation of human dynamin-1 domains. (*bottom left*) Crystal structure of nucleotide-free dynamin-1 lacking its proline-rich domain (PRD). The protein harbors a mutation G397D that blocks the assembly of dimeric dynamin into higher-order oligomers (Ford et al. 2011). (*bottom middle*) Crystal structure of bacterial dynamin-like protein (BDLP) bound to GDP (Low & Lowe 2006) (*right*) and modeled from a cryoelectron reconstitution of GMPPNP-bound BDLP decorating a lipid tube (Low et al. 2009) (*left*). (*bottom right*) Crystal structures of the soluble domain of GDP-bound atlastin-1 (Byrnes & Sondermann 2011). All structures are shown as chain-bows, with colors progressing from cold to hot in an N- to C-terminal direction. (*b*) Schematic representation of the functional domains of the (*top*) yeast and human division and (*bottom*) fusion machinery compared with the prototypical fission DRP, dynamin-1, and the minimal fusion DRP, atlastin-1. Abbreviations: aa, amino acids; BSE, bundle signaling element; GD, GTPase domain; GED, GTPase effector domain; MD, middle domain; PH, pleckstrin homology.


a
PH
BSE
GD
BSE
MD
Insert B
GED
BSE
PRD
Dynamin-1
BDLP
Alastin-1
BSE
GD
Middle domain
+ GED stalk
Insert B (PH)
b
Division
Dynamin-1
864 aa
Yeast
Human
Dnm1
757 aa
DRP1
699–736 aa
Alternative splicing
Mdv1
715 aa
MFF
342 aa
Caf4
643 aa
MiD49
454 aa
MiD51
463 aa
Fis1
115 aa
FIS1
152 aa
Fusion
Atlastin-1
558 aa
Yeast
Human
Fzo1
855 aa
MFN1
741 aa
MFN2
757 aa
MPP processing
Pcp1 processing
MPP processing
S1/S2 processing
Mgm1
881 aa
Opa1
960–1,015 aa
Ugo1
502 aa
Alternative splicing
GTPase domain
Middle domain
Insert B
GTPase effector domain
Proline-rich domain
Bundle signaling element
Heptade repeats
Transmembrane domain
WD40 repeats
Coiled-coil domain
Tetratricopeptide repeat domain
Nucleotidyl transferase domain
Mitochondrial targeting sequence
Hydrophobic region

DRP1, the stalk mediates the formation of an obligate dimer and two additional interfaces, which mediate dimer-dimer interactions (Faelber et al. 2011, Ford et al. 2011, Frohlich et al. 2013). In contrast, the mitochondrial fusion DRPs OPA1 and Mgm1 are not obligate dimers, but they also assemble via their respective stalks into higher-order fusion-promoting structures that may not be helical (Ban et al. 2010, DeVay et al. 2009).

Minimal DRP domain structures have illuminated another critical and common DRP assembly interface: a dimer interface between G-G domains that varies between members, but whose formation is GTP-dependent and required for assembly-stimulated GTP hydrolysis (Chappie et al. 2009, 2010). G-G interface formation is regulated in many DRPs by a distinct three-helix-bundle structural element located proximal to the GTPase domain, termed the bundle signaling element (BSE). The BSE is composed of helices from the N and C termini of the G domain and the C terminus of the GED. In dynamin, GTP binding facilitates G-G dimer formation across helical rungs by changing the orientation of the BSE relative to the G domain to a more open conformation (Chappie et al. 2011). Conformational changes in the G-BSE created by the GTP cycle are likely transduced to the stalk for membrane scission. Indeed, the stalk of bacterial dynamin-like protein (BDLP) transitions from a closed to an open conformation in a GTP-dependent manner, consistent with the possibility that the stalk also transduces GTP-dependent conformational changes in other DRPs (Low & Lowe 2006, Low et al. 2009). Not all DRPs have a defined BSE, such as atlastin, a distantly related DRP that catalyzes homotypic ER fusion (Bian et al. 2011, Byrnes & Sondermann 2011, Liu et al. 2012b, Morin-Leisk et al. 2011, Moss et al. 2011, Orso et al. 2009). In atlastin, the G domain is flexibly linked to the stalk via proline residues, and different atlastin structures indicate that a similar conformation change alters the relative orientation of the G-stalk domains. These changes in atlastin in the context of a G-G dimer that forms across membranes, as opposed to helical rungs, have been proposed to generate force for membrane tethering during fusion.

The insert B region is the most highly variable between DRP members and is situated at the base of the stalk. In dynamin, this region forms a pleckstrin homology domain that binds to PtdIns-4,5 P2 (PIP45P2) lipids to mediate plasma membrane targeting and likely contributes to membrane scission by sensing high membrane curvature and inserting into the membrane bilayer (Ferguson et al. 1994, Mehrotra et al. 2014, Ramachandran et al. 2009). Similarly, the inner membrane fusion DRPs, Mgm1 and OPA1, are selectively targeted to the inner membrane via their respective insert B regions, which bind cardiolipin (CL), a unique mitochondrial lipid that is made and highly enriched in the inner membrane (Ban et al. 2010, DeVay et al. 2009, Rujiviphat et al. 2009). As in atlastin, in the mitochondrial outer membrane fusion DRPs, Fzo1/MFNs, this region is composed of a set of transmembrane helices that anchor these proteins into the outer membrane and are also critical determinants of membrane fusion (Hermann et al. 1998, Liu et al. 2012b, Moss et al. 2011, Rapaport et al. 1998). The insert B region in the mitochondrial division DRPs, Dnm1 and DRP1, also likely binds lipids, but this region has also been proposed to directly mediate interactions with mitochondrial outer membrane proteins responsible for division DRP targeting (Bui et al. 2012, Gallego et al. 2010, Strack & Cribbs 2012). Evidence suggests that, in addition to its role in targeting, insert B regions are responsible for the regulation of stalk-driven assembly through an occlusion mechanism (Kenniston & Lemmon 2010, Mehrotra et al. 2014, Ramachandran et al. 2009, Strack & Cribbs 2012). Consistent with this idea, insert B regions are hot spots for regulation in the form of splice variance and posttranslational modifications and for human disease-linked mutations (for example, see Cribbs & Strack 2007, Kenniston & Lemmon 2010, Strack et al. 2013).

Thus, a common core mechanism for DRP membrane remodeling is emerging that exploits the modular nature of DRP architecture for the opposing processes of membrane scission and

fusion. Membrane scission DRPs may share the ability to form helical structures to mediate membrane scission via G-G interactions between adjacent rungs. In contrast, the fusion DRP atlastin is thought to drive membrane fusion via interactions between G-G domains across membranes. Consistent with the idea of a *trans* interaction, both Fzo1 and MFNs, which have domain architecture similar to that of atlastin, are required on both mitochondrial partners for mitochondrial outer membrane fusion (Hoppins et al. 2011b, Meeusen et al. 2004). However, the MFN heptad repeat (HR) 2 region, which is predicted to comprise part of the stalk domain based on the structure of the related BDLP, forms an antiparallel coiled-coil structure, proposed to tether opposing mitochondrial membranes early during fusion (Koshiba et al. 2004, Low & Lowe 2006, Low et al. 2009). Evidence in yeast suggests that Fzo1 mediates *trans* G-G interactions across membranes (Anton et al. 2011). Thus, future experiments will be needed to determine whether both G-G and HR2 tethering mechanisms are important for mitochondrial outer membrane fusion. Interestingly, the inner membrane fusion DRPs, Mgm1/OPA1, possess a domain architecture that is more related to scission DRPs. In this context, OPA1 is not required on both mitochondrial partners for inner membrane fusion (Hoppins et al. 2011b, Song et al. 2009). These observations raise the possibility that the DRP mechanisms for outer and inner membrane fusion are fundamentally different.

## Mitochondrial Fusion: Mechanisms and Physiological Roles

Little is understood about how the activities of the outer and inner membrane mitochondrial fusion DRPs are coordinately regulated at the molecular level. Data from both cell-based and in vitro fusion assays indicate that although mitochondrial outer and inner membrane fusion events are often coordinated in cells, they are separable and mechanistically distinct (Legros et al. 2002, Meeusen et al. 2004). This raises the interesting possibility that outer and inner membrane dynamics are separately controlled and functionally differentiated. In this context, the action of DRP1 on the outer membrane can mediate inner membrane scission without outer membrane scission (Labrousse et al. 1999, Shim et al. 2012). The inner membrane structure formed from such events may be separately resolved via the action of the inner membrane fusion machine alone. Such an independent inner membrane dynamics pathway would likely be important for super organization of the organelle.

In yeast, the non-DRP outer membrane protein, Ugo1, is essential for fusion and, based on circumstantial evidence, has been proposed to coordinate outer and inner membrane fusion (Coonrod et al. 2007; Sesaki & Jensen 2001, 2004; Wong et al. 2003). Ugo1 has been proposed to function in the fusion of each mitochondrial membrane at a step after membrane tethering and needs to be present on only one mitochondrial partner (Anton et al. 2011, Hoppins et al. 2009). Ugo1 physically interacts with both Fzo1 and Mgm1, and the interaction between Fzo1 and Mgm1 requires Ugo1 (Sesaki & Jensen 2004, Wong et al. 2003). Although the exact molecular role of Ugo1 has not been resolved, data suggest that it can act as an Fzo1 effector to facilitate its assembly in a GTP-dependent manner, to promote tethering of mitochondrial outer membranes (Anton et al. 2011). Ugo1 belongs to the mitochondrial carrier protein family, whose members are responsible for transporting various molecules, including fatty acids, across the inner membrane (Belenkiy et al. 2000). As such, Ugo1 may facilitate lipid mixing at the site of fusion by directly modulating the lipid and/or chemical environment (Hoppins et al. 2009). In this context, in mammals, a mitochondrial-anchored phospholipase D enzyme, MitoPLD, facilitates MFN-mediated mitochondrial fusion through the generation of phosphatidic acid (PA) via the hydrolysis of CL (Choi et al. 2006). Thus, MitoPLD and Ugo1 may serve to directly modify bilayer structure and/or produce a lipid mark to facilitate the spatial regulation of Fzo1/MFN-mediated membrane fusion. Mammals also possess outer membrane-localized carrier-like proteins, such as MTCH2, which

functions during apoptosis to facilitate the activation of the proapoptotic Bcl2 family member, Bax (Palmieri 2013, Robinson et al. 2012). Future work is needed to determine whether they, like Ugo1, also function in mitochondrial dynamics.

The yeast F-box protein, Mdm30, is also a regulatory fusion component (Anton et al. 2011, Cohen et al. 2011, Dürr et al. 2006, Escobar-Henriques et al. 2006, Fritz et al. 2003, Neutzner & Youle 2005). It is proposed to function after Ugo1 to ubiquitylate Fzo1 following GTP hydrolysis, potentially driving membrane fusion via Fzo1 proteasomal degradation. Under mitochondrial stress, K63 ubiquitylation of the mammalian MFNs by the E3 ligase Parkin has also been reported, which leads to proteasome-dependent degradation of MFN and subsequent inhibition of mitochondrial fusion during the process of mitophagy (Chan et al. 2011, Chen & Dorn 2013, Gegg et al. 2010, Ziviani et al. 2010). In other cases, MFN ubiquitylation may also promote fusion, as a recently identified small molecule inhibitor of the mitochondrial deubiquitinase enzyme, USP30, increased ubiquitylation of MFNs and promoted MFN-mediated fusion in a manner not dependent on proteolytic turnover (Yue et al. 2014). Thus, a role of ubiquitin as a posttranslational regulator of mitochondrial fusion may be conserved, but the exact mechanistic mode likely varies.

The mechanistic significance of two Fzo1-like proteins, MFN1 and MFN2, in mammalian cells is not clear. Evidence suggests that although there is some redundancy, they are also specialized. In vitro, the formation of tethered mitochondria occurs more readily with mitochondria from cells overexpressing MFN1 (Ishihara et al. 2004), and only MFN2 has been shown to associate with the Bcl2 proteins, Bax and Bak, which, in addition to regulating cell death, also alter MFN2-dependent fusion (Hoppins et al. 2011b, Karbowski et al. 2006). Pathogenic mutations have been reported only in MFN2 and result in CMT syndrome 2A2 (CMT2A2), a peripheral neuropathy associated with axonal degeneration of neurons with long axonal projections (Kijima et al. 2005, Zuchner et al. 2006). $MFN2^{CMT2A2}$ mutations can be complemented in cells by the formation of Mfn1–$Mfn2^{CMT2A2}$ hetero-oligomers but not homo-oligomers of $Mfn2^{+}$–$Mfn2^{CMT2A2}$ (Detmer & Chan 2007). This observation provides insight into the tissue specificity of CMT2A2 as the relative levels of MFN2 and MFN1 expression vary in tissues and indicates that the control of the expression levels of each protein serves to regulate mitochondrial dynamics in a tissue-specific manner. The sensitivity of this type of neuron to loss of MFN2 underscores an important link between mitochondrial dynamics and motility. Indeed, MFN2 is required for the transport of axonal mitochondria and has been reported to associate with the Miro/TRAK/Milton complex, which regulates mitochondrial motility (Baloh et al. 2007, Misko et al. 2010).

The mitochondrial inner membrane DRPs, Mgm1 and OPA1, are regulated by divergent proteolytic mechanisms. Proteolytic processing of Mgm1/OPA1 generates long isoforms, N-terminally anchored in the inner membrane, and short, soluble isoforms in the intermembrane space (Duvezin-Caubet et al. 2006, Esser et al. 2002, Griparic et al. 2007, Herlan et al. 2003, Ishihara et al. 2006, Song et al. 2007). Functional and biochemical studies indicate that long- and short-OPA1/Mgm1 isoforms assemble together to mediate efficient fusion but are functionally nonredundant, similar to the MFNs and bacterial DRPs (DeVay et al. 2009, Meeusen et al. 2006, Zick et al. 2009). Processing of Mgm1 is mediated by the rhomboid protease, Pcp1, at a site whose accessibility responds to mitochondrial ATP levels, thereby coupling fusion to bioenergetic status (Herlan et al. 2004). OPA1 biogenesis is more complicated, influenced at the transcriptional level, where splicing yields a total of eight variants whose relative functions are unknown. Splice variation dictates the mode of OPA1 proteolytic processing. OPA1 exons 5 and 5b introduce two distinct cleavage sites, S1 and S2, used by the metalloprotease OMA1 and the intermembrane space AAA protease, YME1L, respectively. YME1L mediates the constitutive processing of OPA1, presumably during its import and sorting, but it is possible that processing is linked to the fusion event per se (Griparic et al. 2007, Ishihara et al. 2006, Song et al. 2007). In contrast, OMA1

cleaves at the OPA1 S1 site in a postsorting mode under conditions of mitochondrial stress, such as depolarization, leading to the conversion of long-OPA1 isoforms to short-OPA1, inhibition of fusion, and mitochondrial fragmentation (Baker et al. 2014, Duvezin-Caubet et al. 2006, Guillery et al. 2008, Ishihara et al. 2006, Song et al. 2007). In this manner, OMA1 functions as a stress integrator that regulates metabolism and promotes mitophagy and cell death (Baker et al. 2014, Quirós et al. 2012). Recent data suggest that the long-OPA1 isoform is sufficient to mediate efficient fusion and that short-OPA1 isoforms may independently function to promote stress-induced mitochondrial division (Anand et al. 2014). This represents a new way of thinking about the roles of OPA1 isoforms in mitochondrial dynamics and points to the importance of balance between the long and short isoforms for homeostasis.

Physiologically, one of the most fundamental roles of mitochondrial fusion is in the mixing of mitochondrial components (Chen et al. 2003, Eura et al. 2003, Legros et al. 2002, Nunnari et al. 1997) (**Figure 2*a***). Content transfer also occurs during transient kiss-and-run mitochondrial merging events (Liu et al. 2009). Although cristae content appears to be preserved during mitochondrial fusion (Wilkens et al. 2013), content mixing can complement defective mitochondria through the redistribution of mitochondrial DNA, mRNA, and proteins. Indeed, there is increased mitochondrial heterogeneity in MFN1/2- and OPA1-null cells, which lose membrane potential in a subset of organelles (Chen et al. 2003, 2005). Intriguingly, although cells singly deficient in either MFN1 or MFN2 show a fragmented phenotype, they still retain residual fusion activity and escape major mitochondrial dysfunction (Chen et al. 2005), suggesting that it is the intermixing of mitochondrial content, rather than the tubular network itself, that is essential for maintaining respiratory capacity.

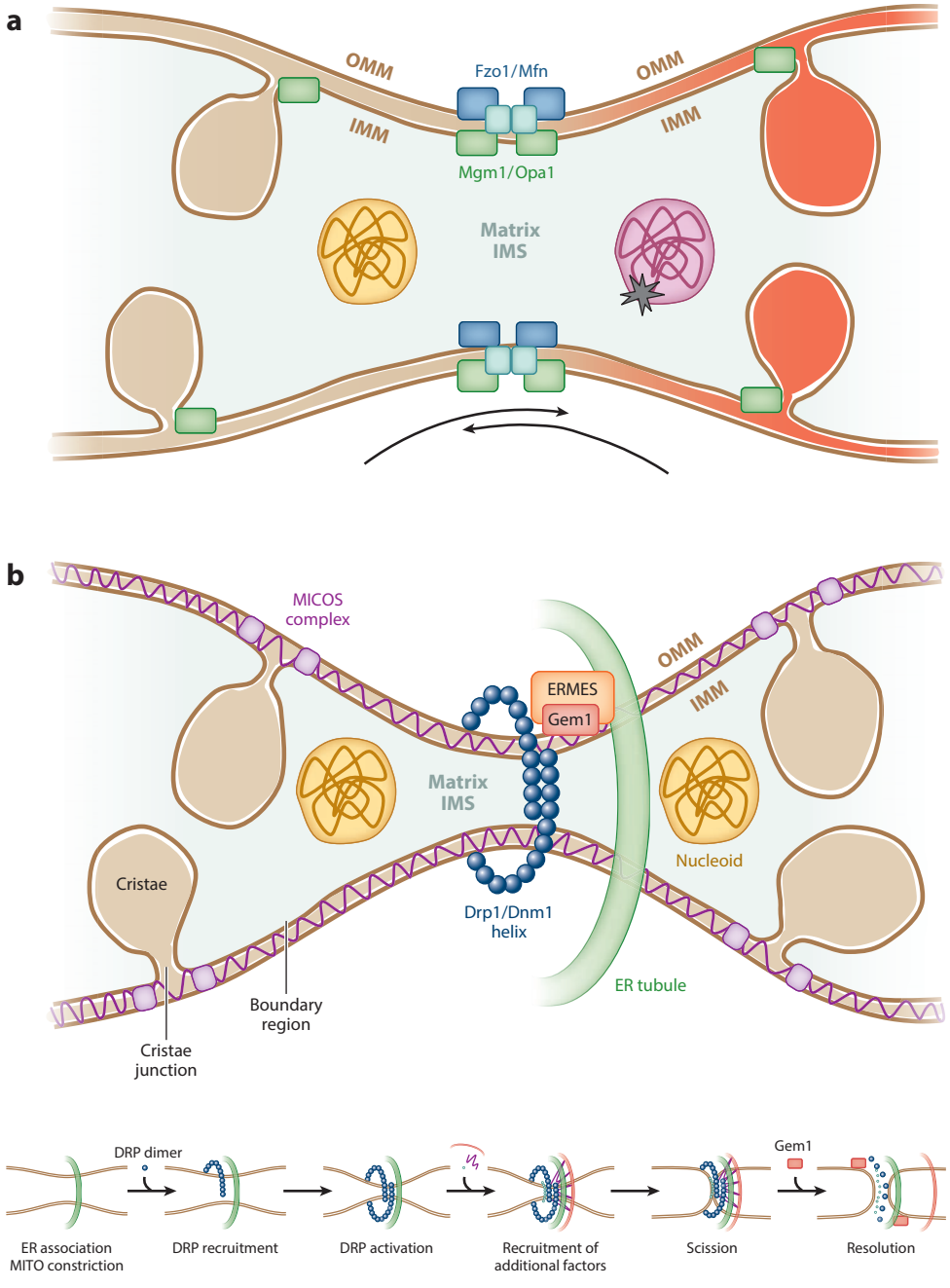
The basis of impaired respiratory function in fusion-deficient cells is not understood, but the current dogma is that it is secondary to the loss of mtDNA. In both yeast and mammals, loss of fusion results in lost mitochondrial genomes. Yeast lacking Fzo1 or Mgm1 are devoid of mtDNA (Hermann et al. 1998, Rapaport et al. 1998), cells lacking OPA1 or both MFN1 and MFN2 contain only one-third of the normal mtDNA levels, and many mitochondria lack nucleoids (Chen et al. 2003, 2005). Fusion is also thought to be important for maintaining mtDNA integrity and replication fidelity. In a muscle-specific MFN1/2-null mouse, there is, in addition to mitochondrial genome depletion, increased frequency of point mutations and deletions in muscle mtDNA, which occur before any phenotypic changes are detected (Chen et al. 2010).

Unlike the tightly regulated transmission of nuclear DNA, mtDNA inheritance is stochastic, or relaxed, in nature, leading to daughter cells acquiring different populations of mtDNA and to unequal segregation of genomes within mitochondria (Birky 1994). In the case of somatic mtDNA mutations, relaxed segregation leads to the coexistence of both wild-type and mutant mtDNA within the same cell and at varying ratios between cells, termed heteroplasmy. Below a given threshold, mutant mtDNA in a heteroplasmic state is tolerated (Nakada et al. 2001), but this depends critically on fusion. Mice carrying an error-prone mtDNA polymerase are viable but cannot survive a combined reduction in fusion through MFN1 deletion (Chen et al. 2010). Consistently, a hybrid formed from two mutant HeLa cell lines carrying pathogenic mutations in different mitochondrial tRNA genes has normal respiratory activity as a result of fusion and content mixing (Ono et al. 2001).

## Mitochondrial Division: Mechanisms and Physiological Roles

The best characterized system for mitochondrial division regulation is the yeast *Saccharomyces cerevisiae*. The yeast division machinery consists of the DRP Dnm1; a C-tail-anchored outer membrane protein, Fis1; and an adaptor-like WD-containing protein, Mdv1. The cytosolic N

terminus of Fis1 forms a tetratricopeptide repeat–like domain that interacts directly with Mdv1 (Dohm et al. 2004, Karren et al. 2005, Mozdy et al. 2000, Suzuki et al. 2003, Tieu & Nunnari 2000, Tieu et al. 2002). Mdv1 functions as a molecular bridge between mitochondrial-anchored Fis1 and soluble Dnm1, and together Fis1 and Mdv1 function to target Dnm1 to the mitochondrial surface (Bui et al. 2012, Cerveny & Jensen 2003, Cerveny et al. 2001, Tieu & Nunnari 2000,



Tieu et al. 2002, Zhang et al. 2012). Mdv1 also functions as a regulator of mitochondrial division post-targeting, to nucleate the assembly of Dnm1 on the mitochondrial surface (Lackner et al. 2009, Naylor et al. 2006). Indeed, biochemical and cytological evidence suggests that the native yeast division machine is a helical structure composed of coassembled Dnm1 and Mdv1 (Lackner et al. 2009). Caf4 is an Mdv1 paralog that also interacts with Dnm1 but whose role in division is minimal (Griffin et al. 2005, Guo et al. 2012). Recent data indicate that Caf4 interacts with Dnm1 at the Num1 mitochondrial tether, which positions mitochondria at the mother cell cortex during cell division, as discussed below (Lackner et al. 2013, Schauss et al. 2006). Although the functional significance of Dnm1 at Num1 tethers is not clear, its unique dependence on Caf4 suggests that Caf4 functions as a different type of effector to differentiate Dnm1 activity at Num1-tethering sites from its activity at mitochondrial division sites. Indeed, DRPs in general are likely to be regulated by both positive and negative effectors.

In mammalian cells, several non-DRP proteins have been identified as receptors for DRP1 targeting to mitochondria; however, their precise regulatory roles have not yet been determined. A structural Mdv1 ortholog is not apparent in higher eukaryotes, and at least four integral outer membrane proteins have been implicated as DRP1 receptors, namely, hFIS1, homologous to the yeast division component, MFF, MiD49, and MiD51 (Gandre-Babbe & van der Bliek 2008, Palmer et al. 2011, Stojanovski et al. 2004, Yoon et al. 2003, Zhao et al. 2011). Evidence for a direct role of hFIS1 in mitochondrial division is controversial, but independent roles for MFF, MiD49, and MiD51 in mitochondrial DRP1 targeting are undisputed (Koirala et al. 2013, Liu et al. 2013, Losón et al. 2013, Palmer et al. 2013). Although MFF promotes DRP1 recruitment and division activity, the role of the MiDs in DRP1 recruitment may not be directly linked with its activation. Overexpression of either MiD49 or MiD51 accumulates and sequesters DRP1 on the mitochondrial surface, resulting in an inhibition of division and formation of a fused mitochondrial network. A hint of the molecular basis of MiD-mediated DRP1 inactivation comes from the observation that MiD overexpression results in the accumulation of the S637 phosphorylated form of DRP1 on mitochondria (Losón et al. 2013). Protein kinase A (PKA) targeted to mitochondria via mitochondrial A-kinase anchoring protein (AKAP1) has been shown to inhibit DRP1 function via S637 phosphorylation, resulting in DRP1 behavior that mimics that seen in a GTP hydrolysis–deficient DRP mutant (Cereghetti et al. 2008, Chang & Blackstone 2007, Cribbs & Strack 2007). Reactivation of S637-phosphorylated DRP1 is mediated by phosphatases, such as calcineurin and mitochondrial protein phosphatase 2A, that, along with PKA/AKAP1, coordinate mitochondrial division with signaling activities, such as neuronal development and cell death (Kim

**Figure 2**

Mechanistic models of mitochondrial fusion and division. (*a*) Schematic of mitochondrial fusion protein topology and interactions diagraming mixing of content and compensation between healthy (*left*, *beige*) and dysfunctional (*right*, *red*) mitochondria. (*b*) Schematic of mitochondrial division machinery at endoplasmic reticulum (ER)-mitochondria contacts. ER-mitochondrial encounter structure (ERMES) and the conserved Miro GTPase Gem1 spatially and functionally link ER-associated mitochondrial division (ERMD) to nucleoid segregation. The mitochondrial contact site (MICOS) complex is depicted as purple waves in the intermembrane space. Below, a schematic model of mechanistically distinct steps in mitochondrial division: ER-mitochondria contact initiates mitochondrial constriction potentially through an actin-based mechanism; DRP recruitment and activation through mitochondrial receptors to mediate DRP helical assembly and assembly-stimulated GTP hydrolysis; recruitment of additional factors, such as myosin II, to complete scission of outer and inner membranes; and resolution of ERMD to link segregation of mitochondria and mtDNA. Abbreviations: IMM, inner mitochondrial membrane; IMS, intermembrane space; OMM, outer mitochondrial membrane.

et al. 2011, Merrill et al. 2013, Slupe et al. 2013). Together, these observations suggest that MiD proteins function as negative effectors of DRP1 to create a pool of mitochondrial DRP1 responsive to specific triggers. In this context, the recently reported structure of MiD51 indicates that it possesses a nucleotidyl transferase domain that binds GDP and ADP, as well as an independent surface loop required for DRP1 targeting (Losón et al. 2014, Richter et al. 2014). In vitro, MiD51 inhibits DRP1 assembly and GTP hydrolysis, and ADP relieves this inhibition, suggesting that this may be relevant for regulation in cells (Losón et al. 2014). In addition to phosphorylation by PKA, DRP1 is posttranslationally modified by ubiquitylation, sumoylation, nitrosylation, and phosphorylation by additional kinases (reviewed in Wilson et al. 2013). Many of these modifications in DRP1 are located in the variable insert B region, which is subject to splice variant modification, further evidence of the regulatory significance of the domain within DRPs. The interplay between multisite posttranslational DRP1 modifications and DRP1 effectors/receptors in the regulation of mitochondrial homeostasis under normal and pathophysiological conditions is thus highly complex and will require further work to elucidate.

## Endoplasmic Reticulum–Associated Mitochondrial Division: Link to mtDNA Distribution

The evolution of DRP-mediated mitochondrial dynamics raises the question of what division site mechanism has replaced the bacterial Min system, which places the FtsZ-like division apparatus at mid-cell to ensure faithful chromosome segregation. In eukaryotes, mitochondrial division site placement is determined by an interorganellar interaction with the ER (Friedman et al. 2011). Prior to DRP recruitment, ER tubules wrap around mitochondria and mark sites of mitochondrial division, a process termed ER-associated mitochondrial division (ERMD), which is conserved from yeast to humans (**Figure 2*b***). Beyond division site placement, ERMD may function to create a geometric hot spot for the assembly of the division DRP helix, where MFF is selectively recruited to efficiently target activated DRP1 in a spatially restricted manner. Such a microdomain might also modulate mitochondrial composition at ERMD sites to directly facilitate or recruit additional factors on the outside and/or inside of mitochondria and promote mitochondrial division. In mammalian cells, there is evidence for actin polymerization at the site of ERMD, mediated via the ER-localized isoform of the formin INF2, and data indicate that myosin II is subsequently recruited, suggesting that an actinomyosin mechanism may facilitate mitochondrial constriction during division (Korobova et al. 2014).

The molecular basis of ERMD is best understood in yeast, where the multiprotein ER-mitochondrial encounter structure (ERMES) is present at sites of ERMD and is required to initiate ER-mitochondria contact. The ERMES complex tethers ER and mitochondria and is composed of five subunits: Mdm10, 12, and 34 and Mmm1, which are each core subunits necessary for ERMES assembly, and Gem1, which is associated with ERMES at steady state, though not required for ERMES assembly (Kornmann & Walter 2010, Kornmann et al. 2011, Richter et al. 2014). Mmm1 is an integral ER protein, whereas Mdm10 and Mdm34 are integral to the mitochondrial outer membrane and Mdm12 is cytosolic. Mdm12, Mdm34, and Mmm1 have synaptotagmin-like mitochondrial-lipid-binding protein (SMP) domains that are predicted to bind and/or facilitate lipid transport, a function that may be intimately related to its role in ERMD (Kopec et al. 2010). Mdm10, in contrast, is a β-barrel protein that also functions as a component of the outer membrane sorting and assembly machinery complex (Meisinger et al. 2004, 2007; Yamano et al. 2010a,b). Gem1 is a highly conserved Miro GTPase that harbors two GTPase domains flanking two EF-hand motifs and is tail-anchored into the mitochondrial outer membrane (Frederick et al. 2004). Although they are present in their ancestors, there are no obvious homologs of ERMES

proteins in animals, indicating that the components may have significantly diverged at the sequence level or were replaced by other ER-mitochondria tethering complexes (Wideman et al. 2013).

ERMES forms a discrete and finite number of interfaces between the ER and mitochondria (Kornmann et al. 2009, Murley et al. 2013). In addition to marking sites of division, ERMES structures are tightly linked to a subset of nucleoids engaged in replicating mtDNA (Hobbs et al. 2001, Meeusen & Nunnari 2003). At sites of ERMD, nucleoids segregate by an unknown mechanism and, in a majority of cases, are distributed to both tips of divided mitochondria (Murley et al. 2013). Deletion of core ERMES components disrupts nucleoid structure and results in a loss of mitochondrial genomes (Boldogh et al. 2003, Hanekamp et al. 2002, Youngman et al. 2004), in addition to causing dramatic effects on mitochondrial morphology. In mammalian cells, nucleoids also localize at mitochondrial tips (Garrido et al. 2003). Silencing of DRP1 leads to cells with large areas of hyperfused mitochondria devoid of nucleoids and the formation of large aberrant nucleoid structures (Ban-Ishihara et al. 2013, Parone et al. 2006), suggesting that ERMD plays a fundamental role in coordinating division with the distribution of the replicating nucleoid. In this context, ERMES has also been implicated as a bridge between mitochondria and the actin network, suggesting that it may serve to link and coordinately drive nucleoid segregation, mitochondrial constriction during division, and mitochondrial distribution after division (Boldogh et al. 2003). Thus, the process of ERMD and nucleoid segregation in yeast may fundamentally be related to the role of actin in ERMD in mammalian cells.

During division, Gem1 is required for the distribution of daughter mitochondria following ERMD (Murley et al. 2013). The metazoan orthologs, Miro1/2, regulate mitochondrial motility, and Gem1 may function similarly, by recruiting yeast-specific motility factors to mitochondrial tips to promote the resolution of daughter mitochondria following division. However, it is also possible that Gem1's role in ERMD is more specific to the nucleoid. Consistent with this, Miro homologs are absent in organisms that lack mtDNA (Vlahou et al. 2011), and *Δgem1* cells rapidly lose mtDNA (Frederick et al. 2004). Whether Miro1 and Miro2 play a similar role in mitochondrial division and mtDNA segregation is not known.

Although division site placement involves the ER, it is not understood how placement of ER-mitochondria contacts is achieved. These questions are related to whether, in a manner analogous to bacterial FtsZ, there is a machine inside of mitochondria that facilitates mitochondrial division. Indeed, nucleoid proteins required for mtDNA maintenance remain localized to discrete punctate structures within mitochondrial tubules in the absence of mtDNA, suggesting that there may be an internal mark associated with mitochondrial division (Meeusen & Nunnari 2003, Spelbrink et al. 2001). In yeast, an excellent candidate for an internal membrane scission machine is the inner membrane protein, Mdm33, which possesses matrix-localized coiled-coil regions that could act in *trans* across inner membranes to mediate constriction (Messerschmitt et al. 2003).

## INTERNAL DETERMINANTS OF MITOCHONDRIAL BEHAVIOR

An internal mark for the placement of mitochondrial division sites might be related to the presence of recently identified mitochondrial skeletal scaffold structures that serve to define domains and higher-level organization of the organelle to integrate many of its functions. Mitochondrial scaffolds include the conserved prohibitin complex, which forms ringlike structures in the inner membrane; the mitochondrial lipids, CL and phosphatidylethanolamine (PE); and the mitochondrial respiratory complexes themselves (Osman et al. 2011, Stuart 2008). Another primary skeletal element in mitochondria is the conserved multisubunit inner membrane associated complex, MICOS (also called MitOS and MINOS) (Harner et al. 2011, Hoppins et al. 2011a, von der

Malsburg et al. 2011). Evidence indicates that MICOS forms an extended heteromorphic structure that organizes and potentially shapes the mitochondrial inner membrane, which is differentiated into at least three regions that are structurally, compositionally, and functionally distinct (refer to **Figure 2*b***). The region closely opposed to the outer membrane is termed the boundary region and possesses the machinery required for lipid trafficking, mitochondrial protein import, and respiratory complex assembly. The inner membrane cristae are invaginated into flat lamellar structures with highly curved edges stabilized by the dimerization/multimerization of ATP synthase complexes and house assembled respiratory complexes (Davies et al. 2012). Relatively narrow tubules, termed cristae junctions, connect cristae to the boundary and partition intermembrane space components, such as cytochrome c, from the boundary. Consistent with its role in organizing mitochondrial membrane domains, MICOS also serves to facilitate mitochondrial biogenesis by interacting with components of the import and sorting machineries in the outer mitochondrial membrane (von der Malsburg et al. 2011). Super-resolution imaging has revealed that mammalian nucleoids are tightly associated with inner membrane cristae (Brown et al. 2011). Thus, these mitochondrial scaffolds, as well as the mitochondrial respiratory complexes themselves, may play important roles in nucleoid positioning and segregation. Consistent with this possibility, elements of the MICOS structure appear adjacent to nucleoids, and loss of an intact MICOS complex leads to nucleoid aggregation (Itoh et al. 2013). How MICOS interacts and is integrated with other scaffolding components to create higher mitochondrial organization is an outstanding question.

## MOTILITY AND TETHERING: POSITIONING AND INHERITANCE INTEGRATED WITH DYNAMICS

The overall behavior of mitochondria is also determined by mechanisms that actively transport and tether mitochondria at defined positions in cells. In yeast, a portion of the mitochondrial network is transported in a directed manner via an actin-based mechanism into the daughter bud of dividing cells (Simon et al. 1997, Yang et al. 1999). Transport is driven in part by the class V myosin motor, Myo2, as mutant *myo2* cells have defects in daughter cell mitochondrial distribution (Altmann et al. 2008; Fortsch et al. 2011; Itoh et al. 2002, 2004), as do cells lacking Ypt11, a Rab-type GTPase reported to interact with Myo2 in directing mitochondrial inheritance (Itoh et al. 2002, Lewandowska et al. 2013), or Mmr1, an outer membrane protein that functions as a cargo adaptor protein for Myo2 recruitment to mitochondria (Chernyakov et al. 2013, Eves et al. 2012, Itoh et al. 2004). Whether this machinery works as a complex to actively transport mitochondria to the bud, or whether components function to tether the network or other recruitment factors within the daughter cell, is still a matter of debate (Boldogh et al. 2004, Frederick et al. 2008, Shepard et al. 2003, Swayne et al. 2011). Additional transport mechanisms likely exist, as movement is delayed but not blocked in Ypt11- or Mmr1-deficient cells (Itoh et al. 2002, 2004). A nonmotor Arp2/3 transport complex has been proposed to function via ERMES and transport mitochondria through the force generated by actin polymerization (Boldogh et al. 2001, Fehrenbacher et al. 2005). In metazoans, in contrast, mitochondria are primarily transported on microtubules via kinesin and dynein-based motility mechanisms, which have been proposed to function together in a complex(es) with Miro1/2 and the adaptor protein Milton/TRAK (as reviewed by Fransson et al. 2006, Glater et al. 2006, Guo et al. 2005, Misko et al. 2010, Wang & Schwarz 2009, Wang et al. 2011). Thus, although the Miro GTPase family is remarkably conserved, the cytoskeletal mode of mitochondrial transport is divergent in yeast and metazoans, whereas in the *Dictyostelium* amoeba, Miro is not required for microtubule-dependent mitochondrial transport (Vlahou et al. 2011). This raises the possibility that the role of Miro in mitochondrial transport is more complex,

perhaps in negotiating the contact between the mitochondria and ER during motility (Friedman et al. 2010).

The ER has also been implicated as a core component of mitochondrial tethering complexes (Lackner et al. 2013, Swayne et al. 2011). In yeast, there are two ER-linked mitochondrial tethers. During cell division, mitochondria in daughter cells are anchored to the cortical ER by Mmr1 (Itoh et al. 2004, Swayne et al. 2011), whose mRNA (Shepard et al. 2003) and protein (Swayne et al. 2011) are actively targeted to mitochondria-ER contact sites in the growing bud. How the Mmr1 tethering role relates to its role as a transport adaptor is unclear and may reflect two distinct, but coordinated functions for the protein. In opposition, in the mother cell, mitochondria are retained by the cortex-associated protein Num1 (Farkasovsky & Küntzel 1995, Heil-Chapdelaine et al. 2000, Klecker et al. 2013, Lackner et al. 2013). Num1 functions antagonistically to Mmr1 to maintain the distribution of mitochondria in both mother and daughter cells, as there is a strong positive interaction between the *NUM1* and *MMR1* genes (Hoppins et al. 2011a) and deletion of *NUM1* rescues the inheritance defect of *Δmmr1* cells (Klecker et al. 2013). Num1 is a large, multidomain protein, which contains a putative pleckstrin homology domain that selectively interacts with plasma membrane PIP lipids and is essential for the formation of an extended, multisubunit anchor that links mitochondria, ER, and the plasma membrane/cortex of the cell (Lackner et al. 2013). Although the ER is involved in both Num1- and Mmr1-dependent mitochondrial tethering, it functions independently of ERMES or ERMD, indicating that the tethering and dynamics machineries function in parallel pathways to control mitochondrial distribution. Although these activities are independent, future work will be needed to unravel how they are integrated at the systems level to adapt to cellular needs. In most metazoan cell types, mitochondrial tethers have not been characterized. The exception is in neurons, where the outer membrane protein syntaphilin facilitates the immobilization of axonal mitochondria at active terminals (Chen & Sheng 2013). Syntaphilin has been suggested to function as a mitochondrial brake, using at least two separate mechanisms (Chen & Sheng 2013). In vitro, it binds directly to the microtubule-based kinesin motor, KIF5, and inhibits its motor activity, suggesting that it converts KIF5 into a component of a static microtubule-dependent mitochondrial tether. Syntaphilin also competes for binding with the kinesin mitochondrial adaptor Milton/TRAK to indirectly facilitate tethering. Whether the ER plays a role in the biogenesis of the syntaphilin/KIF5 tether as it does in yeast tethering complexes is an outstanding question. However, in both systems there is extensive interplay between the motility and tethering machines to control mitochondrial distribution in an activity- and spatially specific manner.

## MITOCHONDRIAL LIPID HOMEOSTASIS

Mitochondrial-ER contact sites likely impinge on many aspects of mitochondrial biology by regulating the distribution of lipids to coordinate the activities of proteins involved in mitochondrial dynamics and positioning, structure, and function. Consistent with this, many of the molecular components that directly regulate mitochondrial dynamics, tethering, and motility possess either integral membrane domains or domains that respond to and are recruited by specific lipid species. Thus, mitochondrial lipid homeostasis dramatically impacts the organization and behavior of mitochondria.

Mitochondria have high proportions of the nonbilayer-forming lipids CL and PE, which are made in mitochondria with precursors transported from the ER and are critical for many mitochondrial functions. CL is made from PA in the inner mitochondrial membrane, whereas the biosynthesis of PE can occur through the Kennedy pathway or by the decarboxylation of phosphatidylserine (PS) in mitochondria. The latter pathway contributes most of the PE found in

mitochondria because PE generated by the Kennedy pathway is poorly incorporated into the organelle (Birner et al. 2001, Chan & McQuibban 2012). In yeast, defects in CL and PE homeostasis are synthetically lethal with loss of protein complexes that control inner membrane structure, such as the prohibitins (Osman et al. 2009) and the MitOS complex (Hoppins et al. 2011a). CL and PE can laterally segregate with one another in membranes (Mileykovskaya et al. 2001), and it is speculated that this ability, in cooperation with prohibitins, creates microdomains that restrict the movement of lipids and proteins within mitochondria (Osman et al. 2009). CL and PE also contribute to assembly of mitochondrial respiratory complexes, protein import machinery, and the processing and activity of Mgm1/OPA1 (Chan & McQuibban 2012, DeVay et al. 2009, Osman et al. 2009, Sesaki et al. 2006). Turnover of CL to PA on the mitochondrial outer membrane by MitoPLD, as previously discussed, is required for mitochondrial fusion (Choi et al. 2006), indicating that turnover of lipids and remodeling of membrane composition are important for mitochondrial fusion.

Like loss of mitochondrial membrane potential, altered lipid distribution, especially of CL, is thought to communicate mitochondrial dysfunction. CL is predominately in the inner membrane in cristae where it binds to cytochrome c and at inner-outer membrane contact sites. Translocation of CL to the OM by phospholipid scramblase 3 is involved in autophagic destruction of mitochondria and apoptosis by targeting LC3 and tBid, respectively (Chu et al. 2013, Lutter et al. 2000). Peroxidation of CL during oxidative stress reduces its affinity for cytochrome c and sensitizes cells to apoptosis (Choi et al. 2006). CL might also contribute to apoptosis by regulating assembly of OPA1 to facilitate cristae remodeling for efficient release of intermembrane space components in apoptosis (Frezza et al. 2006). Sphingolipid metabolites, such as ceramide, also contribute to apoptosis by stimulating Bax/Bak oligomerization (Chipuk et al. 2012) or by directly forming channels in the outer membrane (Colombini 2010), but the mechanism of sphingolipid trafficking to mitochondria is not known.

ER-mitochondria contact sites are crucial for lipid exchange between the two organelles. The ERMES complex might facilitate exchanges simply by bringing the two organelles close together (Kornmann et al. 2009, Nguyen et al. 2012), by binding lipids through ERMES protein lipid–binding SMP domains (Kopec et al. 2010), or by bringing sites of membrane curvature together (Toulmay & Prinz 2012). However, the role of ERMES in lipid exchange is controversial: Several groups have reported altered mitochondrial lipid profiles in ERMES mutants (Kornmann et al. 2009, Osman et al. 2009, Tan et al. 2013b), whereas other groups have seen little effect of ERMES on mitochondrial lipid profiles or ER-mitochondria lipid-exchange rates (Nguyen et al. 2012, Voss et al. 2012). Alternative pathways for phospholipid entry into mitochondria might be activated in ERMES mutants, thus leading to differences in results based on different strain backgrounds, genetic suppression, or nongenetic adaptation.

The conserved PRELI proteins Ups1 and Ups2 function antagonistically to maintain proper ratios of CL and PE in mitochondria. Ups1 (PRELI in humans) and Mdm35 (TRIAP1 in humans) complexes transfer PA to the inner membrane, where it is converted to CL (Connerth et al. 2012, Potting et al. 2013). In contrast, Ups2 is not involved in trafficking of PS to the inner membrane (Tamura et al. 2012), and the lower PE in *ups2* mitochondria is instead probably caused by accelerated export of PE (Osman et al. 2009). The biochemical mechanism of Ups2 remains unknown. Mdm35 forms independent complexes with Ups1 and Ups2 and is critical for stabilizing both proteins, protecting them from proteolysis by Yme1, and regulating the relative abundance of CL and PE in mitochondria (Potting et al. 2013). In mammals, PRELI-TRIAP1 complexes are a target of both cell-survival and cell-death stimuli. TRIAP1 is positively regulated by p53 (Felix et al. 2009), and PRELI-TRIAP1-dependent transport of PA and accumulation of CL in the inner

membrane are important for apoptosis resistance (Potting et al. 2013). Proapoptotic stimuli cause degradation of PRELI-TRIAP1 complexes, leading to reduced mitochondrial CL and enhanced intermembrane space protein release (Potting et al. 2013). Thus, cell-survival signals enhance mitochondrial CL synthesis, whereas cell-death signals inhibit it. Important challenges going forward are to better understand ER-mitochondria lipid trafficking in mammals, the function of ERMES in lipid exchange, the mechanisms of Ups2 and related proteins, the function of trace mitochondrial lipids, and the spatial distribution of lipids within mitochondria.

## MITOCHONDRIAL BEHAVIOR IS INTEGRATED WITH SIGNALING PATHWAYS: MITOPHAGY AND CELL DEATH AS PARADIGMS

Mitochondrial dynamics have evolved to coordinate the structure and function of the mitochondrial network with the bioenergetics needs of the cell. Mitochondrial behavior is thus highly integrated with cellular processes, aiding in the regulation of cellular homeostasis and contributing acute responses to stress (**Figure 3**). The ER appears to play a major role in regulating mitochondrial stress responses by coordinating the machinery of mitochondrial dynamics with that of signaling pathways.

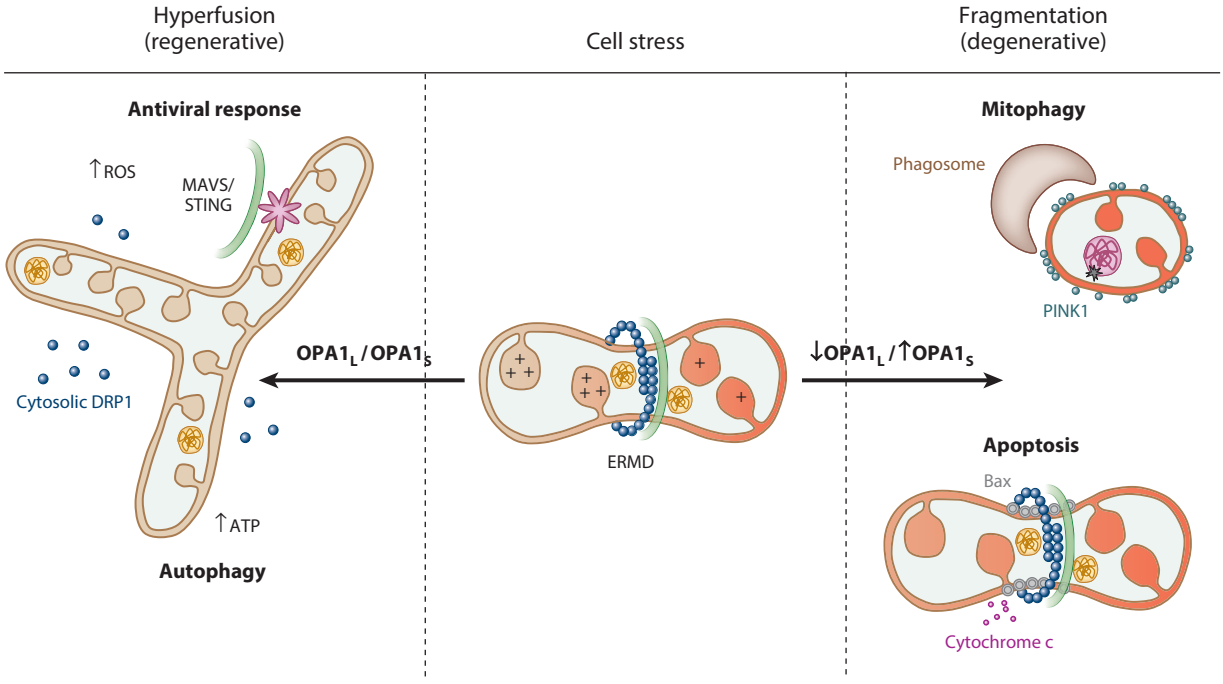



**Figure 3**

Mitochondrial stress response pathways are integrated with mitochondrial dynamics. (*right*) Loss of membrane potential in dysfunctional mitochondria leads to impaired processing of OPA1, loss of fusion activity, and mitochondrial fragmentation, which favors destructive stress responses, such as mitophagy and apoptosis. Stabilization of PINK1 on the membrane of depolarized mitochondria induces mitophagy. Oligomerization of Bax at sites of DRP1 accumulation, which likely represent ERMD microdomains, induces MOMP and cytochrome c release. (*left*) In stress conditions where mitochondrial function must be preserved, such as autophagy and immunity, inhibition of DRP1 recruitment promotes mitochondrial hyperfusion, allowing the organelles to maintain ATP production and adapt bioenergetics to cellular needs.

In conditions of starvation or metabolic stress, cells upregulate autophagy, a conserved catabolic process responsible for the breakdown of cytoplasmic and organellar components. In a process orchestrated by the ATG proteins, intracellular material is captured within a double membrane autophagosome and transported to the lysosome for degradation, providing macromolecular precursors for anabolic processes and serving in a quality-control capacity to eliminate dysfunctional cellular components (Rabinowitz & White 2010). In mammalian cells, starvation results in elongated, tubular mitochondria (Gomes et al. 2011, Rambold et al. 2011). The rapid increase in cAMP in starving cells activates PKA, which phosphorylates Drp1 at S637, preventing its recruitment to the outer membrane (Gomes et al. 2011). This hyperfusion also depends on the activities of OPA1, MFN1, and the inner membrane protein SLP2, but not of MFN2 (Tondera et al. 2009). Hyperfusion spares mitochondria from degradation (Gomes et al. 2011, Rolland et al. 2009). Indeed, mitochondria are a late autophagy substrate (Eiyama et al. 2013, Kristensen et al. 2008), suggesting their maintenance allows the cell to maintain stable ATP production and meet metabolic needs while other materials are recycled (Gomes et al. 2011, Tondera et al. 2009). Interestingly, in *S. cerevisiae*, mitochondrial respiratory deficiency suppresses autophagy during amino acid starvation, possibly because it is no longer energetically beneficial to degrade cellular content when ATP can be solely generated by glycolysis (Graef & Nunnari 2011). Hyperfusion likely represents an effort to preserve ATP levels, a more general response to stress (Tondera et al. 2009). Consistent with this idea, in *Caenorhabditis elegans*, mitochondrial hyperfusion is a transient response that compensates for complex IV deficiency caused by a reduction in the matrix ribonucleoprotein-binding protein, LRPPRC (Rolland et al. 2013). Hyperfusion is also observed during antiviral immunity and is required for an efficient host response (Castanier et al. 2010, Horner et al. 2011, Koshiba et al. 2011, Onoguchi et al. 2010).

Coordination of mitochondrial behavior with autophagy also occurs through contact with the ER, which plays a determinative role in autophagosome biogenesis and cargo. The cellular origin of autophagosomes has been debated (Axe et al. 2008, Hailey et al. 2010, Hamasaki et al. 2013, Hayashi-Nishino et al. 2009, Moreau et al. 2011, Ravikumar et al. 2010, Suzuki et al. 2001, van der Vaart et al. 2010), but several recent studies have defined ER-to-Golgi transport components as key determinants (Ge et al. 2013, Graef et al. 2013, Suzuki et al. 2013, Tan et al. 2013a). In both yeast and mammalian cells, autophagosome formation was functionally and spatially associated with ER exit sites and the COPII vesicle formation machinery (Graef et al. 2013, Suzuki et al. 2013, Tan et al. 2013a). Membrane fractionation of a cell-free assay for mammalian phagophore initiation further identified the ER-Golgi intermediate compartment as a primary autophagosome membrane source (Ge et al. 2013). Together with the finding that the autophagy-specific TRAPPIII complex binds and tethers COPII vesicles to sites of autophagosome formation in a manner similar to TRAPPI tethering of ER-derived COPII coated vesicles to acceptor membranes (Tan et al. 2013a), these studies suggest that during starvation, COPII vesicles are specified to an autophagic biogenesis pathway that is parallel and orthologous to the secretory pathway. In this way, the ER is ideally positioned to integrate the autophagic response with the physiology of other organelles, including the mitochondria. The preautophagosomal marker ATG14 was reported to mark the site of autophagosome formation at areas of ER-mitochondrial contact (Hamasaki et al. 2013), and tomography and fluorescence microscopy have shown transient colocalization of autophagosomes with the outer membrane (Hailey et al. 2010). The mitochondria may also contribute directly to autophagosome formation, as fluorescent PE generated from an ER-derived PS analog was reported to transfer from mitochondrial to autophagosomal membranes (Hailey et al. 2010).

Autophagy selective for mitochondrial elimination, or mitophagy, is used as a quality-control mechanism, presumably to target dysfunctional organelles under steady-state conditions (Kissova et al. 2004, Schweers et al. 2007, Tal et al. 2007). Contrary to autophagy, mitophagy is associated with mitochondrial fragmentation (Nowikovsky et al. 2007, Twig et al. 2008). A balance away from fusion may serve to produce smaller mitochondria that can be engulfed by autophagosomes and also functions to isolate damaged mitochondria from the rest of the network, as mitochondria with lower membrane potential are less fusion competent (Twig et al. 2008). In mammalian cells, depolarization directly inhibits mitochondrial fusion by enhancing OPA1 processing via OMA1 activation (Duvezin-Caubet et al. 2006, Griparic et al. 2007, Ishihara et al. 2006, Song et al. 2007), and in yeast, low matrix ATP levels result in the loss of the short-Mgm1 isoform and accumulation of the long (Herlan et al. 2004). Conversely, loss of mitochondrial division impairs mitophagy and causes an accumulation of oxidized mitochondrial proteins, supporting the idea that dynamics is linked to mitochondrial quality control (Arnoult et al. 2005b, Gomes & Scorrano 2008, Twig et al. 2008).

In mammalian cells, mitochondria are targeted for degradation by the PINK1/Parkin ubiquitylation pathway (Youle & Narendra 2011). In healthy cells, the protein kinase PINK1 is constitutively imported from the cytosol into the intermembrane space, where it is cleaved by the inner membrane protease PARL and subsequently degraded (Deas et al. 2011, Jin et al. 2010, Lin & Kang 2008, Yamano & Youle 2013). Loss of membrane potential stabilizes PINK1 at the outer membrane, where it recruits and activates Parkin, an E3 ubiquitin ligase. The Parkin-dependent K63 ubiquitylation of several proteins on the mitochondrial outer membrane leads to their proteasomal degradation and the recruitment of autophagic machinery, resulting in mitophagy (Geisler et al. 2010, Kim et al. 2008, Matsuda et al. 2010, Narendra et al. 2010, Vives-Bauza et al. 2010). The outer membrane MFN and Miro proteins are targets for Parkin-mediated degradation, which causes mitochondrial fragmentation and loss of motility to facilitate mitochondrial segregation and elimination (Liu et al. 2012a, Wang et al. 2011, Weihofen et al. 2009). Mutations in PINK1 and Parkin are linked to early-onset familial Parkinson's disease, suggesting that PINK1/Parkin-mediated mitophagy is critical for the maintenance of normal mitochondrial function in cells, especially dopaminergic neurons (Youle & Narendra 2011).

In addition to their canonical roles in regulating mitochondrial structure, the mitochondrial division and fusion DRPs function to regulate apoptosis, a form of programmed cell death initiated by developmental cues or intracellular stresses. In vertebrates, these intrinsic apoptotic signals converge on mitochondria through the activation of Bcl-2 homology 3–only proteins, causing the oligomerization of proapoptotic members of the Bcl-2 family, Bax and Bak, on the outer membrane into foci that are linked to outer membrane permeabilization (MOMP) and the release of cytochrome c and other death factors to initiate apoptotic caspase activation in the cytosol (Chipuk et al. 2010, Vaux 2011). During apoptosis, DRP1 plays a positive regulatory role in MOMP. DRP1 is recruited and activated at the mitochondrial outer membrane via posttranslational modifications, causing a dramatic fragmentation of the mitochondrial network (Frank et al. 2001, Wasiak et al. 2007). Fragmentation is not required for apoptosis, however (Estaquier & Arnoult 2007, Parone et al. 2006), suggesting that the role of mitochondrial division and fusion proteins in cell death is more direct and not a consequence of changes in mitochondrial shape per se (Karbowski et al. 2004, Lee et al. 2004, Neuspiel et al. 2005, Olichon et al. 2003, Sugioka et al. 2004). The exact mechanism by which mitochondrial DRPs influence MOMP is not understood. OPA1 appears to play a role in the reorganization of cristae junctions that occurs during apoptosis, allowing the redistribution of cytochrome c from the cristae folds into the inner membrane space for release during MOMP (Frezza et al. 2006, Scorrano et al. 2002). These morphological changes

are associated with disruption of the oligomeric state of OPA1 (Arnoult et al. 2005a, Cipolat et al. 2006, Frezza et al. 2006, Yamaguchi et al. 2008). During apoptosis, both DRP1 and MFN2 are found in foci colocalized with Bax on mitochondria (Karbowski et al. 2002). These apoptotic foci spatially mark mitochondrial constriction sites and mitochondrial tips, consistent with the idea that they are associated with the observed increase in mitochondrial division and fragmentation.

The connection of mitochondrial division with MOMP has raised the possibility that MOMP is spatially linked to ERMD sites and that mitochondrial DRPs regulate MOMP by influencing the shape and/or composition of ER-mitochondria microdomains. Consistent with this, DRP1 was shown to promote tBid-induced Bax oligomerization in CL-containing membranes by stabilizing membrane tethering and lipid mixing through hemifusion intermediates (Montessuit et al. 2010). Such hemifusion events are thought to occur during mitochondrial fission and may form at CL-rich outer and inner membrane contact sites (Ardail et al. 1990). In addition, ER-derived sphingolipid metabolites have been shown to act as cofactors for Bax/Bak activation at the outer membrane (Chipuk et al. 2012). Tailoring of local lipid composition through ER-mitochondria exchanges may also allow the coordination of diverse apoptotic signaling elements (Gonzalvez et al. 2008, Kuwana et al. 2002, Lutter et al. 2000). Movement of $Ca^{2+}$ from the ER to mitochondria is another critical apoptogenic process that is facilitated by close apposition of the two membranes, by establishing local regions of high $Ca^{2+}$ concentration that trigger mitochondrial transporters, $Ca^{2+}$ uptake, overload, fragmentation, and MOMP (Csordas et al. 2006, Filippin et al. 2003, Pinton et al. 2008). In this way, ER-mitochondrial contacts may allow for the tailoring of signaling microdomains in a context-dependent manner. Such a specialized signaling site is also seen during antiviral responses. Inflammatory and antiviral pathways are transmitted through the formation of an outer membrane signaling complex that involves interactions between the mitochondrial membrane protein, MAVS (Dixit & Kagan 2013), and the ER integral protein, STING (Ishikawa & Barber 2008, Jin et al. 2008, Sun et al. 2009, Zhong et al. 2008). Although interaction with division machinery has not been reported, the MAVS/STING complex colocalizes with the ER, which may, as for apoptosis, provide the specific protein, lipid, and ionic conditions required for signaling.

The adaptor protein hFIS1 may play a key role in coupling mitochondrial dynamics with stress responses and in integrating these signals with the ER. In response to mitochondrial $Ca^{2+}$ influx from the ER, FIS1 and DRP1 mediate a fragmentation response that, if prolonged, is associated with cytochrome c release and apoptosis (Hom et al. 2007). During apoptosis, FIS1 induces mitochondrial fragmentation and interacts with the integral ER protein Bap31 to stimulate its cleavage into the proapoptotic p20 fragment, promoting MOMP and cell death (Breckenridge et al. 2003, Iwasawa et al. 2011). In healthy cells, overexpression of Fis1 leads to fragmented mitochondria and spontaneous mitophagy (Gomes & Scorrano 2008), a response that seems to be a result of widespread mitochondrial dysfunction, rather than a direct fission-dependent event (Alirol et al. 2006, Gomes & Scorrano 2008). Conversely, deletion of Fis1 in *C. elegans* and mammalian cells results in aberrant mitophagy characterized by large mitophagosome aggregates (Shen et al. 2014). This defect is specific to mitophagy, as starvation-induced autophagosome formation is unaffected and is dependent on Parkin and DRP1. In stress conditions, FIS1 was found to bind phospho-S600 DRP1 in a complex with the ER proteins calnexin and Bap31. In the absence of Fis1, mitophagosomes form elongated structures along microtubules (Yamano et al. 2014). These formations do not accumulate in $Mff^{-/-}$ or $Drp1^{-/-}$ cells, further indicating that fission per se does not account for FIS1's function in stress pathways. More recent data indicate that FIS1 is a scaffold for TBC1D15, a mitochondrial Rab GTPase–activating protein, and functions at the interface between mitochondria and autophagosomes to regulate Rab7-dependent autophagosomal growth around mitochondria during mitophagy (Lee et al. 2004, Stojanovski et al. 2004, Suzuki et al. 2003).

FIS1 thus appears to play a role in coordinating stress machinery with mitochondrial dynamics (Otera et al. 2010).

## SUMMARY POINTS

1. The opposing processes of mitochondrial fusion and division are mediated by a family of highly conserved large dynamin-related GTPase proteins (DRPs) that function through nucleotide-dependent self-assembly and hydrolysis.
2. DRP activities are controlled at many levels by interaction regulatory factors, posttranslational modifications, proteolytic processing, and alternative splicing.
3. Fusion and division work in concert with internal scaffold-like structures as well as transport and tethering machineries to regulate mitochondrial behavior and distribute mitochondrial genomes throughout the mitochondrial network.
4. ER-mitochondria contact sites create functional microdomains. Interactions with the ER define the site of mitochondrial division and replicating nucleoid segregation. The ER also contributes to mitochondrial tethering independently of its role in division.
5. Mitochondrial lipid homeostasis is critical for the regulation of the activities of proteins involved in dynamics, structure, positioning, and function and likely specifically impacts ER-mitochondria contact sites.
6. Mitochondrial behavior is highly integrated with signaling pathways, such as mitophagy and programmed cell death.

## FUTURE DIRECTIONS AND UNSOLVED ISSUES

1. Mechanistically, how are DRP activities regulated by the accessory factors of the fusion and division machinery? How is this integrated with DRP posttranslational modifications?
2. How is the dynamics machinery coordinated with internal structural components to regulate mitochondrial genome distribution and ultrastructural organization? What role does the ER play?
3. What lipid and protein components define the microdomains formed by ER-mitochondria contact sites? What other cellular factors, such as actin and myosin, are involved in the positioning and function of these sites?
4. What factors fulfill the tethering and lipid transfer functions of ERMES in higher eukaryotes? In this context, do the Miro proteins perform the same function as Gem1? What is the function of ERMES in lipid exchange?
5. How are lipids positioned within mitochondria? How do this spatial distribution and the presence of trace lipids affect mitochondrial behavior? What are the mechanisms of Ups2 and related proteins in mitochondria lipid homeostasis?
6. How are the division and fusion activities of the DRPs coordinated with their roles in signaling? How does the ER contribute to these functions?

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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