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The Endoplasmic Reticulum-Mitochondria Encounter Structure and its Regulatory Proteins

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Abstract

In fungi, the endoplasmic reticulum-mitochondria encounter structure (ERMES) is present between the endoplasmic reticulon (ER) and mitochondria to promote the formation of the ER-mitochondria contact sites. Four constitutive components (Mmm1, Mdm12, Mdm34, and Mdm10) assemble to form the ERMES complex while regulator proteins are required for regulating the organization and function of the ERMES complex. Multiple regulator proteins, including Gem1, Lam6, Tom7, and Emr1, of the ERMES complex, have been identified recently. In this review, we discuss the organization of the ERMES complex and the roles of the regulator proteins of the ERMES complex.

Keywords

ER, ERMES, mitochondria, membrane contact sites

Introduction

Membrane contact sites allow communication between different organelles, promoting the exchange of metabolites, signaling molecules, and other building blocks of life (Lahiri et al., 2015; Lang et al., 2015). The endoplasmic reticulum (ER) forms membrane contact sites with many other membrane-bound organelles, including mitochondria, the Golgi apparatus, peroxisomes, lysosomes, and endosomes (Elbaz & Schuldiner, 2011; Gatta & Levine, 2017; Honscher & Ungermann, 2014). Similarly, mitochondria form contact sites with many membrane-bound organelles, including vacuoles/lysosomes (through vacuole and mitochondria patch, vCLAMP) (Elbaz-Alon et al., 2014), peroxisomes (through peroxisome-mitochondria contact site, PerMit) (Shai et al., 2018), and the ER (through the endoplasmic reticulum-mitochondria encounter structure, ERMES) (Kornmann et al., 2009).

Mitochondria not only serve as the powerhouse of a cell but also are invovled in a wide range of fundamental cellular activities, including amino acid, lipid, and nucleotide metabolism (Spinelli & Haigis, 2018), redox homeostasis (Spinelli & Haigis, 2018), and Ca^{2+} signaling and transport (Rizzuto et al., 2004). The contact sites between the ER and mitochondria have been a focus of intensive studies. In mammalian cells, the ER-mitochondria contact site is referred to as mitochondria-associated membranes (MAMs) (van Vliet et al., 2014). Malfunctions of MAMs lead to neurological pathologies, including amyotrophic lateral sclerosis (ALS), and Parkinson's and Alzheimer's diseases (Area-Gomez et al., 2018; Bernard-Marissal et al., 2018; Giorgi et al., 2015). In yeasts, three lipid transfer proteins, that is, Mmm1, Mdm12, and Mdm34, and a mitochondrial β-barrel protein, that is, Mdm10, are present at the ER-mitochondria interface to form the ERMES complex (AhYoung et al., 2015; Kornmann et al., 2009). Malfunctions of the ERMES complex alter the contact between mitochondria and the ER and mitochondrial morphology, impair mitochondrial functions, and compromise lipid metabolism (Lahiri et al., 2014; Lang et al., 2015). In this review, we discuss the organization of the ERMES complex and summarize the regulatory proteins of the ERMES complex.

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The ERMES complex

The Constitutive Components of the ERMES Complex

The ERMES complex was first discovered in the budding yeast *Saccharomyces cerevisiae* by a genetic screen (Kornmann et al., 2009). In the screen work, the authors engineered a chimera protein (i.e. ChiMERA) capable of tethering mitochondria to the ER (through binding ER membrane and the mitochondrial outer membrane) and used the chimera tether to rescue the lethality associated with impaired ER-mitochondria contacts in mutant cells. This tether-based genetic screen led to the identification of Mdm12 (mitochondrial distribution and morphology 12), a constitutive component of the ERMES complex (Kornmann et al., 2009). In the absence of the chimera tether, *mdm12* mutant cells do not grow on respiration plates. Before the tether-based screen, it has been reported that Mdm12 forms a complex with Mdm10 (mitochondrial distribution and morphology 10) and Mmm1 (maintenance of mitochondrial morphology 1) (Boldogh et al., 2003). Using a combination of yeast genetics and microscopy approaches, Kornmann et al., further revealed that Mdm12, Mdm10, Mmm1, and Mdm34 (mitochondrial distribution and morphology 34) are the constitutive components of the ERMES complex (Kornmann et al., 2009).

Among the four constitutive components, Mdm34 and Mdm10 are mitochondrial outer membrane proteins (Mdm10 is integral to the mitochondrial outer membrane while Mdm34 is peripheral), Mmm1 resides on the ER, and Mdm12 is a cytosolic protein. Generally, the ERMES complex localizes as 1–5 discreet foci in the cytoplasm of a cell but fails to assemble when any of the four constitutive components malfunctions. Before the concept of ERMES was proposed (Kornmann et al., 2009), the four components of the ERMES have been shown to have functions associated with mitochondria. Mmm1 was first identified as a mitochondrial outer membrane protein responsible for regulating mitochondrial morphology since tubular mitochondria become spherical in *mmm1* mutant cells (Burgess et al., 1994). By contrast, it was shown later that Mmm1 is a membrane protein inserted into the ER (Kornmann et al., 2009; Stroud et al., 2011). The cytosolic protein Mdm12 was initially identified as a crucial protein required for proper mitochondrial inheritance (Berger et al., 1997) and was later shown to bridge the ER and mitochondria (Kornmann et al., 2009; Kornmann & Walter, 2010). Mdm34 was reported to be a protein residing on the mitochondrial outer membrane and functioning to maintain mitochondrial morphology and mtDNA (mitochondrial DNA) (Youngman et al., 2004). Similarly, Mdm10 was identified as a β-barrel protein required for regulating mitochondrial morphology (Paschen et al., 2003; Sogo & Yaffe, 1994). In addition, Mdm10 was found to associate with the Sorting and Assembly Machinery (SAM) complex to promote mitochondrial beta-barrel assembly (Meisinger et al., 2007; Thornton et al., 2010; Yamano et al., 2010a).

Studies on the localization interdependence of the ERMES components showed that Mmm1 localizes to the ER in the absence of any of the other three ERMES components while Mdm34 or Mdm10 resides on mitochondria in cells lacking either of the other three ERMES components (Kornmann et al., 2009). Interestingly, the absence of either Mdm34 or Mdm10 causes Mdm12 to localize to the ER while the absence of Mmm1 causes Mdm12 to localize to mitochondria (Kornmann et al., 2009). This result, together with the fact that Mdm12 does not possess a transmembrane domain, indicates that Mdm12 likely functions to bridge the ER and mitochondria through interacting with Mmm1 and Mdm34 or Mdm10.

Architecture Organization of the ERMES Complex

Recently, the architecture organization of the ERMES complex has been characterized extensively (AhYoung et al., 2015;

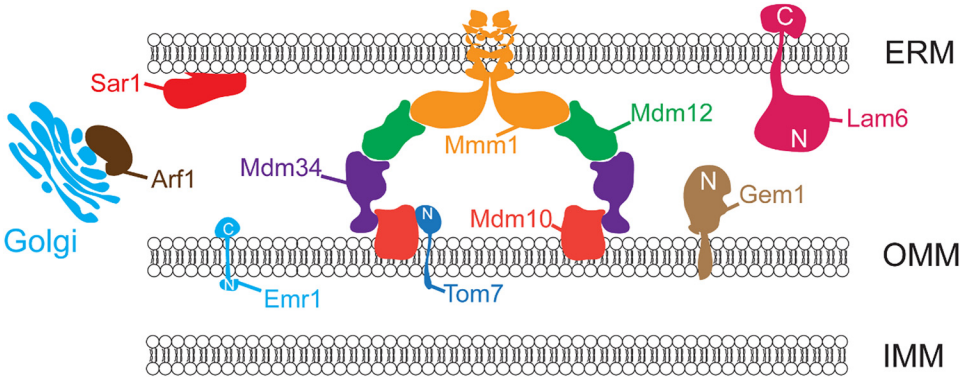

Figure 1. A schematic diagram illustrating the ERMES complex and its regulatory proteins. The ERMES complex is composed of four constitutive components: Mmm1, Mdm12, Mdm34, and Mdm10. A possible organization model of the ERMES complex is shown. Lam6 and Sar1 reside at the ER, Arf1 localizes at the Golgi apparatus, and Gem1, Tom7, and Emr1 localize to the mitochondrial outer membrane. ERM indicates ER membrane while OMM and IMM represent the outer and inner mitochondrial membranes, respectively.

AhYoung et al., 2017; Jeong et al., 2016; Jeong et al., 2017; Kawano et al., 2018). A possible model for the structural organization of the ERMES complex is shown in Figure 1. All ERMES constitutive components except Mdm10 (i.e., Mdm12, Mdm34, and Mmm1) carry an SMP (synaptotagmin-like mitochondrial-lipid-binding protein) domain, which is involved in lipid transport (AhYoung et al., 2015). The structures of the Mmm1 SMP domain and full-length Mdm12 have been determined (AhYoung et al., 2017; Jeong et al., 2016; Jeong et al., 2017; Kawano et al., 2018). In addition, the structure of Mdm10 in complex with the SAM (the sorting and assembly machinery) complex has also been determined recently (Takeda et al., 2021). However, the structure of Mdm34 has not to been solved. According to the structure work, Mmm1 likely forms a homodimer in a head-to-head conformation (Jeong et al., 2017), while Mmm1 and Mdm12 may interact with one another in a tail-to-head configuration to form a hetero-tetramer in equimolecular stoichiometry (AhYoung et al., 2015; Jeong et al., 2016; Jeong et al., 2017). The interaction between Mdm34 and Mdm12 appears to be transient, and thus it is difficult to determine the mode of the Mdm34-Mdm12 interaction structurally (AhYoung et al., 2015). Nonetheless, it was proposed that Mdm34 may interact with Mdm12 through the extreme N-terminal β1-strand adjacent to the SMP domain of Mdm34, since a similar β1-strand adjacent to the SMP domain of Mdm12 is also responsible for forming Mdm12 homodimers and the N-terminal structures of Mdm12 and Mdm34 are similar (Jeong et al., 2016). How the β-barrel protein Mdm10 interacts with Mdm34 and anchors the ERMES complex at the ER-mitochondria contact site is unknown. To understand fully the assembly and function of the ERMES complex, the structure of the entire ERMES complex should be characterized in the future.

The Functions of the ERMES Complex

In addition to tethering the ER to mitochondria, the ERMES complex is involved in several important cellular functions. First, the SMP-containing components of the ERMES complex coordinate to transport lipids between the ER and mitochondria (Kawano et al., 2018). Two possible models, that is, the lipid carrier model and the continuous conduit model, have been proposed to explain the underlying mechanism of lipid transport by the ERMES complex (Kawano et al., 2018). Second, the ERMES complex plays a critical role in maintaining tubular mitochondrial morphology and the inheritance of mtDNA (Berger et al., 1997; Burgess et al., 1994; Sogo & Yaffe, 1994; Youngman et al., 2004). Third, the ERMES complex is involved in regulating mitophagy through ubiquitination of Mdm34 and Mdm12 by the E3 ligase Rsp5 (Belgareh-Touze et al., 2017). Since lipid synthesis plays crucial role in regulating mitochondrial morphology and mitophagy, it is possible that the second and third functions stated above depend on the role of the ERMES complex in lipid transport between the ER and mitochondria. However, it is also possible that ERMES components mediate the second and third functions of the ERMES complex directly.

The Regulatory Proteins of the ERMES complex

Membrane contact sites are generally dynamic in nature, and the size and organization of the membrane contact sites may be regulated in a cell-cycle and/or cell-state-dependent manner (Elbaz-Alon et al., 2015; Honscher et al., 2014; Honscher & Ungermann, 2014). For example, the nuclear vacuolar junction (NVJ) expands during the stationary phase (Pan et al., 2000), and the number of the ERMES foci increases, and the size of vCLAMP decreases when cells are grown in non-fermentable media (Honscher et al., 2014). Therefore, it is conceivable that different types of regulator proteins may be required to regulate membrane contact sites (Scorrano et al., 2019).

Recently, intensive efforts have been directed to identify and characterize the regulatory proteins of the ERMES complex. Nonetheless, only six regulatory proteins have been reported (Figure 1 and Table 1). Among the regulatory proteins, three (i.e. Gem1, Tom7, and Emr1) reside on mitochondria, one (i.e., Arf1) localizes to the Golgi apparatus, and two (i.e., Lam6 and Sar1) reside on the ER. Lam6 and Sar1 are involved in regulating the area of the mitochondria-ER contact site while Arf1, Gem1, Tom7, and Emr1 are involved in regulating the organization of the ERMES complex. It is worth noting that Gem1, Sar1, and Arf1 are GTPases, indicative of the crucial roles of GTPases in regulating the organization of the ERMES complex. Among the four constitutive components of the ERMES complex, only Mdm12 and the SMP domain of Mmm1 are purified successfully (AhYoung et al., 2015; Jeong et al., 2016; Jeong et al., 2017; Kawano et al., 2018). Thus, it has remained difficult to reconstitute the ERMES complex in vitro, and therefore, it is technically challenging to determine the specific roles of the regulatory proteins in regulating the assembly or organization of the ERMES complex in vitro. We summarize the function of the six regulatory proteins in detail below.

Gem1, a Protein Regulating the Number and Size of ERMES Foci

Gem1 was found to be an interacting protein of the ERMES complex (Kornmann et al., 2011; Stroud et al., 2011) and has two Mammalian homologs, that is, Miro-1 and Miro-2 (Mitochondrial Rho) (Fransson et al., 2003). Similar to Gem1, Miro-1 is found to localize to the ER-mitochondria contact site (Kornmann et al., 2011). Gem1 localizes to the outer mitochondrial membrane, depending on a hydrophobic

Table 1. Regulatory Proteins of the ERMES Complex.

Regulator names	Organism	Systematic name	Intracellular localization	Mitochondrial phenotype (null mutant)	ERMES and contact site phenotypes	Reference marker used to study ERMES	Interacting ERMES component	References
Gem1	*Saccharomyces cerevisiae*	YAL048C	Mitochondrial outer membrane	Globular and spherical	Reduced number ERMES foci (null mutant) No effect (null mutant)	Mdm34-mCherry, Mmm1-GFP	Mdm34	(Kornmann et al., 2011) (Nguyen et al., 2012)
Lam6	*Saccharomyces cerevisiae*	YLR072W	ER Membrane and/or Intracellular membrane contact	No significant effect	Increased number of ERMES foci upon overexpression	Mdm34-GFP, Mdm34-mCherry	Mdm34	(Murley et al., 2015) (Elbaz-Alon et al., 2015)
Sar1	*Saccharomyces cerevisiae*	YPL218W	ER Membrane and COPII vesicles	Clusters of mitochondrial fragments	Increased region of contact sites (null mutant)	GFP-Mdm34 Mmm1-RFP	N.D.	(Ackema et al., 2016)
Tom7	*Saccharomyces cerevisiae*	YNL070W	Mitochondrial outer membrane	Aggregated /unevenly distributed mitochondria	N.D.	Mdm10	Mmm1, Mdm12, Mdm10, Mdm34	(Ellenrieder et al., 2016)
Arf1	*Candida albicans*	YDL192W	Golgi	Fragmented	Increased number of ERMES foci (null mutant)	Mmm1-GFP	N.D.	(Zhang et al., 2018)
Emr1	*Saccharomyces pombe*	SPAC8C9.19	Mitochondrial outer membrane	Fragmented and aggregated	Reduced number of ERMES foci but increased ERMES size (null mutant)	Mdm12-tdTomato, Mmm1-tdTomato	Mdm12, Mdm34	(Rasul et al., 2021)

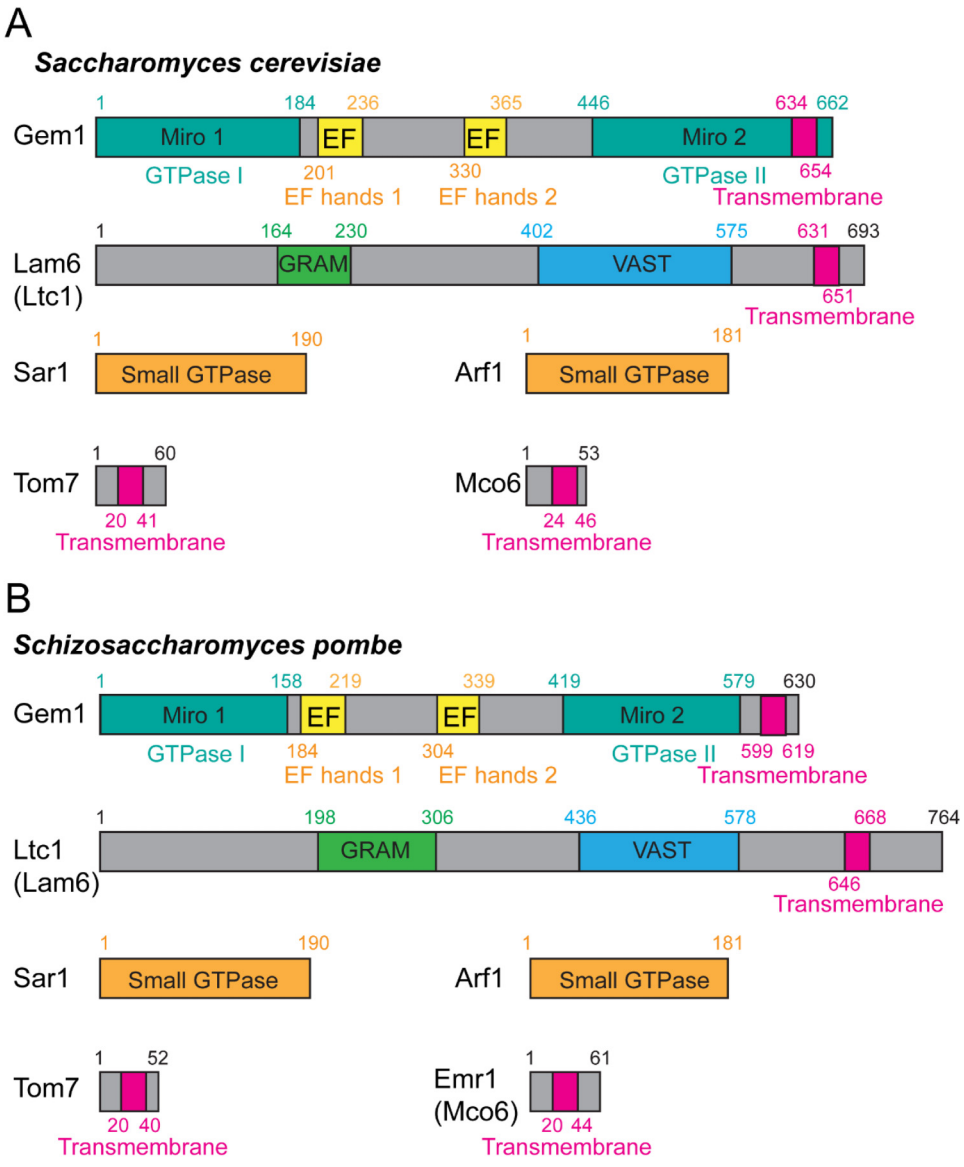

Figure 2. Domain organization of the indicated ERMES interacting proteins in the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe*.

tail at its C-terminus (Stroud et al., 2011). The absence of Gem1 increases the size of the ERMES foci but decreases the number of the ERMES complex (Kornmann et al., 2011). Nevertheless, the ERMES complex is still able to form in the absence of Gem1. These results suggest that Gem1 is not a constitutive component of the ERMES complex and may function as only a regulatory protein of the ERMES complex.

Analysis of the structural organization of Gem1 shows that Gem1 is a special GTPase containing two GTPase domains and two EF-hand domains (capable of binding Ca^{2+}) (Kornmann et al., 2011), and a transmembrane helix is present at the extreme C-terminus of Gem1 required for insertion of Gem1 into the mitochondrial outer membrane (Figure 2). The N-terminal GTPase and EF-hand domains likely dictate the localization of Gem1 to the ERMES foci because mutations in the two domains impair the localization of Gem1 to the ERMES foci (Kornmann et al., 2011). By contrast, the C-terminal GTPase domain does not appear to contribute to the localization of Gem1 to the ERMES complex (Kornmann et al., 2011). Instead, the C-terminal GTPase domain plays a role in lipid biosynthesis (Kornmann et al., 2011). The function of the C-terminal EF-hand domain is unclear.

The absence of Gem1 leads to spherical mitochondria or mitochondrial aggregates, a phenotype similar to the one caused by malfunctions of the ERMES components (Frederick et al., 2004; Kornmann et al., 2009). Therefore, the altered number and size of ERMES foci observed in cells lacking Gem1 could be a secondary effect of abnormal

mitochondria. Alternatively, the altered number and size of ERMES foci are directly caused by the absence of Gem1. Nguyen et al. found that the number and size of ERMES foci are not significantly different in wild-type cells and cells lacking Gem1 (Nguyen et al., 2012). These contradictory findings make the role of Gem1 in regulating the ERMES complex controversial. Similarly, the role of Gem1 in regulating lipid transport between the ER and mitochondria is debatable (Kornmann et al., 2011; Nguyen et al., 2012). By contrast, the crucial role of Gem1 in maintaining mitochondrial morphology is evident. Tethering mitochondria to the ER with an artificial mitochondria-ER tether does not rescue the altered mitochondrial morphology in cells lacking Gem1 (Nguyen et al., 2012). This result indicates that the role of Gem1 in maintaining tubular mitochondrial morphology is independent of its role in regulating the ERMES complex. This interpretation appears to be narrow. It is possible that the ERMES complex may not function as only a tether or as a tether at all. If this is the case, tethering mitochondria to the ER in cells lacking Gem1 with the artificial mitochondria-ER tether would not rescue the mitochondrial phenotype. Therefore, it is possible that Gem1 may maintain tubular mitochondrial morphology by regulating the ERMES complex. Whether and how Gem1 is involved in regulating the organization of the ERMES awaits further characterization.

Lam6/Ltc1, a Versatile Protein Regulating Both ER-Vacuole and ER-Mitochondria Contract Sites

Lam6/Ltc1 was identified as an interacting protein of the ERMES complex by mass spectrometry (Elbaz-Alon et al., 2015; Murley et al., 2015). It has three mammalian homologs, i.e. GRAMD1a, GRAMD1b, and GRAMD1c (Elbaz-Alon et al., 2015; Murley et al., 2015). The absence of Lam6 does not appear to affect the ERMES complex and mitochondrial morphology (Elbaz-Alon et al., 2015; Murley et al., 2015). Moreover, the absence of ERMES components does not affect Lam6 (Elbaz-Alon et al., 2015). However, cells lacking both Lam6 and any of the ERMES components are inviable, indicating that Lam6 and the ERMES complex play a parallel role in regulating cell viability (Elbaz-Alon et al., 2015). Therefore, although Lam6 is an interacting protein of the ERMES complex, Lam6 is not an essential component of the ERMES complex.

Lam6 is an integral ER protein and has been found to be involved in the formation of the junctions between the ER and mitochondria (organized by the ERMES complex), between vacuoles and mitochondria (vCLAMP, vacuole, and mitochondria patch), and between vacuoles and the nucleus (NVJ, nuclear vacuolar junction) (Elbaz-Alon et al., 2015). Disruption of vCLAMP leads to expansion of the ER-mitochondria contact site (i.e., ERMES) in a Lam6-dependent manner (Elbaz-Alon et al., 2015). Similarly, overexpression of Lam6 causes expansion of ERMES, vCLAMP, and NVJ (Elbaz-Alon et al., 2015). Therefore, Lam6 likely plays a crucial role in facilitating the communication between vCLAMP and ERMES.

Analysis of domain structure reveals that Lam6 contains mainly two domains, that is, a GRAM (Glucosyltransferases, Rab-like GTPase activators, and Myotubularins) lipid-binding domain and a VAST (VAD1 analog of StART) domain (Murley et al., 2015) (Figure 2). It has been reported that the GRAM domain plays a role in regulating protein localization (Begley et al., 2003; Doerks et al., 2000). Indeed, the localization of Lam6 to the ER-mitochondria contact site, but not the ER-vacuole contact site, depends on the GRAM domain (Murley et al., 2015). In addition, it has been shown that the VAST domain forms a hydrophobic pocket to accommodate lipid molecules (Khafif et al., 2014). Consistently, the VAST domain of Lam6/Ltc1 is required for binding and/or transporting ergosterol (Murley et al., 2015), and the complex structures of Lam proteins associated with ergosterol have been solved (Tong et al., 2018). By contrast, all SMP domains identified so far bind and transfer phospholipids, but not sterols. Whether Lam6/Ltc1 coordinates with the ERMES complex to mediate lipid transport remains to be tested, and the specific role of Lam6 in regulating the ERMES complex awaits further investigation.

Tom7, a Regulatory Protein Controlling the Shuttle of Mdm10 Between the ERMES and SAM Complexes

Although Tom7 is a component of the TOM complex, it also interacts with the beta-barrel membrane protein Mdm10, a component of the ERMES complex (Yamano et al., 2010b). In addition to being a constitutive component of the ERMES complex, Mdm10 can associate with the sorting and assembly machinery (the SAM complex) and shuttles between the ERMES and SAM complexes (Boldogh et al., 2003; Meisinger et al., 2004; Meisinger et al., 2007; Yamano et al., 2010b). The SAM complex is required for the assembly of the TOM complex (Becker et al., 2011; Paschen et al., 2003). Tom7 and the SAM complex share a similar binding site on Mdm10, and thus binding Tom7 to Mdm10 blocks the interaction of Mdm10 with the SAM complex (Ellenrieder et al., 2016). Since the ERMES complex and Tom7 do not share binding sites on Mdm10, the interaction between Tom7 and Mdm10 enhances the specificity of Mdm10 to the ERMES complex. Consistently, in cells lacking Tom7, Mdm10 dissociates from the ERMES complex and binds the SAM complex, reducing the amount of the Mdm10-containing ERMES complex (Becker et al., 2011; Ellenrieder et al., 2016; Yamano et al., 2010b). The role of Tom7 in controlling the shuttle of Mdm10 between the ERMES and SAM complex has been clearly demonstrated. However, the significance of the dual regulation of Tom7 on the ERMES and SAM complex has not been well understood.

Arf1, an Inhibitory Regulator of ERMES Formation

The small GTPase Arf1 localizes to the Golgi apparatus in *Candida albicans* and has been reported to be a regulator protein of the ERMES complex (Zhang et al., 2018). The absence of Arf1 accumulates cellular reactive oxygen species (ROS) and increases the number of ERMES foci (Zhang et al., 2018). Moreover, clearance of ROS in cells lacking Arf1 attenuates the formation of the ERMES complex (Zhang et al., 2018). These findings suggest that the accumulation of ROS after the deletion of *arf1* could be the cause of the increased number of ERMES foci. However, it is puzzling that induction of ROS accumulation in wild-type cells does not promote the formation of ERMES foci (Zhang et al., 2018). Therefore, how Arf1 is involved in regulating the ERMES complex remains unclear.

The canonical role of Arf1 is to be involved in membrane trafficking (Rabouille, 2014). Nonetheless, Arf1 has been shown to interact with Gem1 genetically in *Saccharomyces cerevisiae* (Ackema et al., 2014). Whether the canonical role of Arf1 in membrane trafficking contributes to regulating the ERMES complex and the ER-mitochondria contact site remains to be tested.

Sar1, a Small GTPase Regulating the Size of the ER-Mitochondria Contact Site

Sar1 is a small GTPase (Figure 2) and is one of the five COPII (Coat protein complex II) proteins, playing a crucial role in the vesicular transport of lipids and proteins between the ER and the Golgi apparatus. Generally, Sar1 is localized to the ER exit sites, a specialized region on the ER for cargo transport from the ER to the Golgi apparatus (Kurokawa et al., 2016). The role of Sar1 in regulating the ER-mitochondria contact site is evident. It has been reported that the N-terminal amphipathic helix of Sar1 has an activity of liposome tubulation, and mutations within the helix (e.g., the mutant N3-Sar1) increase the area of the ER-mitochondria contact site (Ackema et al., 2016); upon activation, Sar1 promotes high membrane curvature, leading to a reduced area of the ER-mitochondria contact site (Ackema et al., 2016). However, how Sar1 executes the role in regulating the ER-mitochondria contact site is unclear. Sar1 may not function through regulating the ERMES complex because the number of ERMES foci is not affected in cells expressing Sar1 mutants (Ackema et al., 2016). Whether the absence of Sar1 affects ERMES foci remains to be tested carefully. In addition, whether and how Sar1 interacts with the ERMES complex is unclear.

Emr1, a Regulatory Protein Dictating the Number of ERMES Foci

We recently identified the outer mitochondrial membrane protein Emr1 (ERMES regulator 1) as a new regulatory protein of the ERMES complex in the fission yeast *Schizosaccharomyces pombe* (Rasul et al., 2021). Similar to the other regulatory proteins of the ERMES complex, the absence of Emr1 does not disrupt the formation of the ERMES complex. Instead, the absence of Emr1 significantly decreases the number of ERMES foci and impairs tubular mitochondrial morphology. Moreover, the size of the ERMES foci in cells lacking Emr1 is larger than the one in wild-type cells. Therefore, Emr1 is not required for the formation of the ERMES complex but plays a crucial role in the organization of the ERMES complex. Although Emr1 has been shown to interact physically with the ERMES components Mdm12 and Mdm34 (Rasul et al., 2021), how the interaction between Emr1 and ERMES components modulates the organization of the ERMES complex is unclear.

Emr1 localizes to the mitochondrial outer membrane, depending on its transmembrane domain in the middle of the protein (Figure 2) (Rasul et al., 2021). Topology and biochemical analyses revealed that the C-terminus of Emr1 is exposed to the cytoplasm, and the C-terminus of Emr1 is an important functional region because the loss of the C terminus fails to rescue the mitochondrial and ERMES phenotypes in cells lacking Emr1 (Rasul et al., 2021). The budding yeast homolog of Emr1 is the uncharacterized protein Mco6, which has a similar domain organization as Emr1 (Figure 2).

Conclusion and Perspective

In fungi, the ERMES complex functions as a crucial tether responsible for the formation of the ER-mitochondria contact site. Therefore, delineation of the dynamic organization of the ERMES complex would facilitate understanding not only the molecular mechanism underlying the formation of the ER-mitochondria contact site but also the function of the ER-mitochondria contact site. Delineation of ERMES organization and functions necessitates the identification and characterization of ERMES regulatory proteins. To date, only a few regulatory proteins, as summarized in this review (Tables 1 and 2), of the ERMES have been identified and characterized. Therefore, one of the attractive directions is to expand the list of the ERMES regulatory proteins and characterize these proteins.

The ERMES complex was found in only fungi so far. Among the four constitutive components of the ERMES complex, Mmm1 has a functional homolog (i.e., PDZD8) in mammalian cells (Hirabayashi et al., 2017). Swapping the Mmm1 SMP domain with that of PDZD8 partially rescues the mitochondria morphology in the *mmm1* mutant (Hirabayashi et al., 2017; Lahiri et al., 2014). Similar to Mmm1, PDZD8 is required for the proper formation of the ER-mitochondria contact site (Hirabayashi et al., 2017; Lahiri et al., 2014). Since PDZD8 is also present at the ER-endosome/lysosome contacts, whether PDZD8 is the specific counterpart of the ERMES complex in mammalian

Table 2. Homologs of the ERMES Regulatory Proteins[a].

Regulator names	*Homo sapiens*	*Saccharomyces cerevisiae*	*Saccharomyces pombe*	*Candida albicans*[b]	*Drosophila melanogaster*	*Caenorhabditis elegans*
Gem1	RHOT1 RHOT2	GEM1	SPCC320.04c	CaO19.13437	Miro	miro-1 miro-2 miro-3
Lam6/ LTC1	GRAMD1A GRAMD1B GRAMD1C	LAM5 YSP2 LAM4 LAM6	SPBC20F10.07	CAALFM_C600890WA CAALFM_C107440WA	GramD1B CG30284	ZC328.3
Sar1	SAR1A SAR1B	SAR1	SPBC31F10.06c	CaO19.10966	Sar1	sar-1
Tom7	TOMM7	TOM7	SPBC27B12.1°c	Tom7p	Tom7	tomm-7
Arf1	ARF1 ARF3 ARF4 ARF5	ARF1 ARF2	SPBC4F6.18c	CaO19.13805 CAL0000178804	Arf79F Arf102F	arf-1.2 arf-3
Emr1	None	MCO6 (YJL127C-B)	SPAC8C9.19	None	None	None

[a]Homologs were searched by DIOPT Ortholog Finder (https://www.flyrnai.org/cgi-bin/DRSC_orthologs.pl), using the gene name of fission yeast.
[b]Homologs for *Candida albicans* were searched by PANTHER (http://www.pantherdb.org/genes/), using the gene name of fission yeast.

cells remains to be further tested. PDZD8 appears to be the sole SMP domain-containing protein at the ER-mitochondria interface. Therefore, it is possible that PDZD8 alone functions as the counterpart of the ERMES complex in mammalian cells. Note that the functional counterparts of the other three ERMES components (i.e., Mdm12, Mdm34, and Mdm10) have not been identified in mammalian cells, which warrants further investigation. In addition, the structural organization of the ERMES complex is still poorly understood due to the technical difficulty in the purification of the ERMES components Mdm34, Mdm10, and Mmm1. Hence, new strategies remain to be developed for reconstitution of the ERMES complex in vitro.

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