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In silico analysis of co-evolution among ERMES proteins, Pex11 and Lam6

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Abstract

In eukaryotic cells communication and dynamic interactions among different organelles are important for maintaining cellular homeostasis. The Endoplasmic-Reticulum (ER) Mitochondria Encounter Structure (ERMES) complex establishes membrane contact sites between ER and mitochondria and is essential for phospholipid transport, protein import and mitochondrial dynamics and inheritance. In this work, *in-silico* analyses were used to probe the intramolecular interactions in ERMES proteins and the interactions that support the ERMES complex. Based on Mutual Information (MI), sites of intramolecular co-evolution are predicted in the core proteins Mmm1, Mdm10, Mdm12, Mdm34, the peroxisomal protein Pex11, and cytoplasmic Lam6; these sites are linked to structural features of the proteins. Intermolecular co-evolution is predicted among the synaptotagmin-like mitochondrial lipid-binding protein (SMP) domains of Mmm1, Mdm12 and Mdm34. Segments of Pex11 and Lam6 also share MI with the SMP domains of Mmm1 and Mdm12, and with the N-terminus of Mdm34, implicating Mdm34 as part of a hub for interactions between ERMES and other complexes. In contrast, evidence of limited intermolecular co-evolution involving the outer membrane protein Mdm10 was detected only with Mmm1 and Pex11. The results support models for the organization of these interacting proteins and suggest roles for Pex11 and Lam6 in regulating complex formation.

Keywords: ERMES, Pex11, Lam6, mitochondria-ER contact sites, mutual information

Introduction

Dynamic interactions among organelles are important for proper functioning of cells. Interactions between mitochondria and the endoplasmic-reticulum (ER) are accomplished in part through a protein complex known as the Endoplasmic-Reticulum Mitochondria Encounter Structure (ERMES, Fig. 1, Kornmann et al. 2009). This complex, which has been detected only in fungi and amoeba to date (Wideman et al. 2013a), participates in the transfer of lipids and proteins, mitochondrial dynamics, mitochondrial DNA inheritance and mitophagy (reviewed in Lang and Peter 2015; Kojima et al. 2016), and new evidence suggests that it is important for fungal immune evasion in *Candida albicans* (Tucey et al. 2016) and virulence of *Aspergillus fumigatus* (Geissel et al. 2017). There are four core components in the ERMES complex: the ER membrane protein Mmm1 (maintenance of mitochondrial morphology), the mitochondrial outer membrane (MOM) protein Mdm10 (mitochondrial distribution and morphology 10), the peripheral protein Mdm34 (also known as Mmm2), and the cytosolic protein Mdm12 (Kornmann et al. 2009; Wideman et al. 2013b).

Mmm1 was first identified in *Saccharomyces cerevisiae* (Burgess et al. 1994). Although this protein resides in the ER (Kornmann et al. 2009), mitochondria of *mmm1Δ* cells have distorted shapes and are defective in transferring mitochondrial DNA to daughter cells (Hobbs et al. 2001). Mmm1 is one of three ERMES complex proteins that contain a synaptotagmin-like mitochondrial lipid-binding protein (SMP) domain (Fig. 2A,B). It is a member of tubular lipid-binding protein (TULIP) superfamily (Kopeck et al. 2010). The N-terminus of Mmm1 resides in the ER lumen and is involved in homodimer formation (Wideman et al. 2013b). Apart from its role in the ERMES complex, Mmm1 has been proposed to play a role in the assembly of a β -barrel protein MOM protein, Tom40 (translocase of the outer membrane 40) (Wideman et al.

2013b).

Mdm12 is a soluble protein (Kornmann and Walter 2010) and *mdm12Δ S. cerevisiae* have giant mitochondria and abnormal mitochondrial distribution (Berger et al. 1997). Like Mmm1, Mdm12 also contains an SMP domain for transporting lipid (Fig. 2A,B) and may form a dimeric structure through a domain swapping mechanism involving an N-terminal β -strand (Jeong et al. 2016). The assembly of some MOM proteins also depends on Mdm12; in an *N. crassa* Mdm12-deletion strain, levels of Tom40, the voltage-dependent anion-selective channel (VDAC), and Mdm10 are low (Wideman et al. 2010).

Mdm34, also known as Mmm2, although initially identified as a MOM protein (Youngman et al. 2004; AhYoung et al. 2015), is a peripheral membrane protein, as revealed by high stringency carbonate extractions of membranes (Ellenrieder et al. 2016). In yeast cells, the absence of this protein causes mitochondrial morphological and growth defects on non-fermentable carbon sources (Youngman et al. 2004) and decreased levels of mitochondrial phosphatidylethanolamine (PE) and cardiolipin (CL) (Wideman et al. 2013b). In contrast, reductions in mitochondrial phosphatidylcholine, PE, CL levels were not detected in a $\Delta mmm2$ strain of *N. crassa* (Wideman et al. 2013b). Expression levels of some mitochondrial proteins such as Tim8 and Tim13 were reduced in this strain, as were those of VDAC, Tom40 and the inner membrane ADP/ATP carrier (Wideman et al. 2013b).

Electron microscopy (AhYoung et al. 2015) and small angle x-ray scattering (AhYoung et al. 2017) data indicate that an elongated, tubular-shaped, hydrophobic, heterotetrameric channel is formed by the SMP domains of Mmm1 and Mdm12 to transport lipid. In this model, Mdm34 is tightly associated with the MOM and through interactions with Mdm12, forms a bridge with Mmm1 and Mdm12 to transport phospholipid between mitochondria and ER (see Fig. 1).

Mdm10 is a β -barrel pore-forming protein (Fig. 2 C,D) in the MOM, and belongs to the Tom40/VDAC superfamily (Bay et al. 2012; Flinner et al. 2013). However, unlike Tom40 and VDAC, Mdm10 is predicted to expose long loops on both sides on the membrane. *N. crassa* mitochondria devoid of Mdm10 have distorted morphology and less efficient assembly of VDAC, Tom40, and Tom22 (Wideman et al. 2013b). In contrast, an *S. cerevisiae* mutant lacking Mdm10 displays decreased levels of Tom40, but an increased amount of VDAC (Meisinger et al. 2007).

Pex11 and Lam6 are two proteins that are proposed to have interactions with the ERMES complex. Lam6, also known as Ltc1, (Murley et al. 2015) is a member of the family of StART-domain lipid transport proteins (Gatta et al. 2015) and may play a regulatory role in sterol transfer and in the assembly and expansion of junctions between mitochondria and ER (ERMES), mitochondria and vacuole (vCLAMP) and nuclei and vacuoles (NVJ, reviewed in Elbaz-Alon et al. 2015; Kojima et al. 2016). It has been proposed that Lam6 interacts with the ERMES complex and aids in the expansion of contact sites under physiological conditions (Elbaz-Alon et al. 2015). Pex11 is a peroxisomal protein that regulates the peroxisome numbers (Mattiuzzi Ušaj et al. 2015). The localization pattern of Pex11 is changed in *mdm10Δ*, *mdm12Δ* and *mdm34Δ* but not *mmm1Δ*, *S. cerevisiae* cells and Pex11 physically interacts with Mdm34 to make contact sites between peroxisomes and mitochondria (Mattiuzzi Ušaj et al. 2015; supplementary table S1).

Thus, the basic organization of the ERMES complex and the interactions of the proteins to perform different physiological activities such as lipid transport have been elucidated. In this study the coevolution of the amino acids in and among different proteins in the ERMES complex was assessed to search for critical groups of residues in the component proteins, and to assess

different ERMES models. These *in-silico* studies may help in future to understand the interacting regions of the proteins and elucidate their physiological roles in more detail.

Materials and Methods

Phylogenetic Analysis

Mdm10 and Mdm31/32 sequences were extracted from online databases NCBI and JGI (MycoCosm, <http://www.ncbi.nlm.nih.gov/>; Grigoriev et al. 2014), aligned using PRALINE (Simossis and Heringa 2005). Evolutionary analyses were conducted in Molecular Evolutionary Genetics Analysis version 6 (MEGA6; Tamura et al. 2013) using the Maximum Likelihood method (ML). Phylogenies were also inferred by Bayesian analysis carried out using the MrBayes program (Ronquist and Huelsenbeck 2003). Details are provided in supplementary file S1.

Mutual information analyses

Unique protein sequences for each of Mmm1, Mdm10, Mdm12, Mdm31/32, Mdm34, Pex11 and Lam6 were obtained from UniProtKB (UniProt Consortium 2015; supplementary table S2), using the UniProt Reference Clusters (UniRef) function to collect sequences with a maximum of 90% identity. Sequences were aligned with ClustalO (Sievers et al. 2011) and manually trimmed to remove any positions with less than 70% identity using MEGA6 (Tamura et al. 2013). These trimmed multiple sequence alignments (MSA) were analyzed for intramolecular MI using MISTIC (Mutual Information Server to Infer Coevolution; Simonetti et al. 2013). The *N. crassa* sequences were used as reference sequences. For intermolecular MI

analysis, MSA were generated using only those sequences for which there was a complete set of all 7 proteins. These MSA were investigated using CAPS2 (Coevolution Analysis using Protein Sequences; Fares and McNally 2006), with the following settings: Bootstrap (BS) 0.95, Gap penalty 0.9, Convergence off. Overlapping groups of coevolving residues are presented. Groups of co-evolving sequences were not predicted for these pairs: Mdm31/Mmm1, Mdm31/Mdm34, Mdm10/Mdm12, Mdm10/Mdm34.

Protein structure predictions

Secondary structures and transmembrane domains were predicted using MSA generated in PRALINE, and the PSIPRED (Jones 1999) and PHOBIUS (Käll et al. 2004) algorithms, respectively, in the PRALINE package (Simossis and Heringa 2005). Protein fold predictions were obtained using the Phyre2 Protein Homology/analogY Recognition Engine V 2.0 (Kelley et al. 2015), using the full-length *N. crassa* sequences. The summary protein modelling data are presented in supplementary table S3.

Results and discussion

To understand the key functional and structural interactions within different ERMES complex proteins and their interactions with other proteins, correlated evolutionary variation and mutual information (MI) data were used to probe the co-evolutionary relationships between two positions, either within one protein, or between two proteins (Fares and McNally 2006, Simonetti et al. 2013). In this work, these relationships were assessed in the context of predicted and known protein structures. The proteins chosen for the study included the core ERMES proteins Mdm10, Mdm12, Mdm34 (Mmm2), Mmm1, (supplementary table S2; AhYoung et al. 2015,

Lang and Peter 2015, Mattiazzi Ušaj et al. 2015, Elbaz-Alon et al. 2015). The Miro GTPase, Gem1, was not included in this study as it is present in substoichiometric amounts and therefore is not considered a component of the core ERMES complex (see Ellenrieder et al. 2016 for discussion). Two other proteins were chosen for study: Lam6 (also referred to as Ltc1), which interacts with ERMES and regulates organellar contacts (Elbaz-Alon et al. 2015) and Pex11, a peroxisomal protein involved in the recruitment of peroxisomes to mitochondria (Mattiazzi Ušaj et al. 2015).

Mdm32, a mitochondrial inner membrane protein, has been proposed to interact with the ERMES complex, based on singlet oxygen triplet energy transfer (STET) experiments (To et al. 2014). Two related mitochondrial inner membrane proteins, Mdm31 and Mdm32, have been identified in *S. cerevisiae* (Dimmer et al. 2005) and both nomenclatures have been used in genome annotations. To determine the relationships among the Mdm31 and Mdm32 proteins, 57 proteins were collected and subjected to phylogenetic analysis (supplementary figure S1). The inferred phylogeny yielded a tree topology with strong bootstrap (node support) values and the Mdm31 sequence groupings appear to reflect the phylogenetic history of the organism from which they were obtained. Mdm32 proteins have been described only in members of the Saccharomycetales and phylogenetic analysis shows that Mdm31 and Mdm32 are monophyletic and these two proteins are most likely paralogues (see nodes 1 and 2; supplementary figure S1). It has been previously suggested that members of the Saccharomycetales including *S. cerevisiae* have undergone a genome duplication event (Wolfe and Shields 1997), and this may have generated the Mdm31 and Mdm32 paralogues. The phylogenetic survey also suggests that Mdm31 paralogs exist among members of the Zygomycota (see nodes 3 and 4; supplementary figure S1). Evidence for co-evolution between Mdm31/32 and other members of the ERMES,

complex was not obtained, and this set of proteins will not be discussed further.

Mmm1

Mmm1 is an ER-anchored protein (see Fig. 1). This protein folds into four α -helices, seven β -strands and an N-terminal transmembrane (TM) domain (residues 18-37), as predicted by PRALINE and Phyre2 (Figs. 2A,B and 3A). Most of these predicted structural components comprise an SMP domain, which generally consists of at least 6 β -stranded segments and 3 α -helices that form a barrel-shaped structure (Fig. 2A,B), the inside of which is mostly composed of hydrophobic residues that forms the tunnel for lipid transport (Reinisch and Camilli 2016, Kojima et al. 2016).

Intramolecular MI analysis shows four regions (1: residues 1-55, 2:88-181, 3:205-242, 4:288-350) with residues potentially co-evolving (Fig. 3A). Region 1 includes cytosolic residues flanking the TM domain and may be involved in the formation of homodimers or assembly in the ER (Wideman et al. 2013b). Residues in this region are suggested to co-evolve with those encompassing the α -helical parts of region 2 and spanning residues 116-133. Residues in region 1 also share MI with sites spanning residues 289-297 in $\alpha 3$ (Fig. 3A). Potential interactions were detected among regions 2 (residues 116-133), 3 (227-242), and 4 (289-301), which presumably are involved with folding and/or stabilization of the SMP domain. It is expected that the overall hydrophobic nature of the tunnel must be maintained and MI involving hydrophobic amino acids may relate to this factor. Notably, the C-terminal section of the protein (residues 351-451 Fig. 3A) is almost devoid of MI, in agreement with its potential role as a flexible hinge.

The residues in the regions flanking the TM are highly conserved among the set of sequences analyzed (Fig. 3A). In terms of the ERMES complex, it is intriguing that several residues on the cytosolic side of the TM domain potentially co-evolved with Mdm34 and Pex11

(Fig. 3A and see supplementary figure S2A for details). Residues in the SMP domain, highlighted in pink in Fig. 3A, share MI with those in Mdm34 and Mdm12 are in line with a recent model for the ERMES complex (AhYoung et al. 2015 and see Fig. 1) and may play important roles for lipid transfer and complex formation. Intermolecular MI also suggests that Pex11 and to a lesser degree, Lam6 and Mdm34, may interact with the outer surface of the SMP domain (supplementary figure S2A). The observation that groups of residues in Mmm1 share MI with those in multiple ERMES proteins may reflect either a network of surfaces that may interact to form the complex, and/or interactions with individual pairs of proteins occur under different conditions or during different stages of assembly of the complex.

Mdm12

Mdm12 is a soluble, SMP-domain containing protein in the ERMES complex, and its folded structure is predicted to contain three α -helices and eight β -strands, forming an SMP fold in the C-terminal part of the protein (Figs. 2 and 3B). Based on intramolecular MI, the residues of Mdm12 from the reference set of sequences can be divided into four regions on the Circos map (1: 1-90, 2: 113-144, 3: 167-260, 4: 493-675). Regions 2 and 3 are predicted to be unstructured, and evidence for intramolecular co-evolution is weaker than that seen for the folded segments. The folded regions are predicted to reside in regions 1 (N-terminal) and 4 (C-terminal) of the protein. As for Mmm1, predicted co-evolution is most notable in the parts of the protein that generate the SMP fold (Fig. 3B). Evidence of co-evolution is detected in region 4, among the segments delimited by residues 628-633, 643-652 and 658-668, and between these residues and those in region 1 (37-52 and 63-75).

In terms of intermolecular co-evolution and structural analysis, Mmm1, Pex11 and Lam6

are predicted to co-evolve with regions in the N- and C-termini of Mdm12 (Fig. 3B and supplementary figure S2B). The partially folded structure predicted for the N-terminus is not part of the SMP fold, but interactions with Pex11 and Lam6 are predicted. Mmm1-Mdm12-Mdm34 physical interactions were proposed in a recent model, in which Mdm34 interacts on the outer surface of the SMP tunnel (AhYoung et al. 2015). β 1, β 2, β 3 and β 4 of Mdm12 co-evolve with residues in Mmm1, suggesting that they form at least part of a surface where the SMP domains of the two proteins can interact to elongate the tunnel. Interactions between Mdm34 and Mdm12 were not suggested by the MI data, but are not excluded by it.

Mdm34

Mdm34 is a MOM-associated SMP-domain containing protein (Youngman et al. 2004; AhYoung et al. 2015; Ellenrieder et al. 2016). The predicted structure of this protein includes four α -helices and three β -strands (Fig. 3C). Based on MI, residues of Mdm34 from the reference set of sequences can be divided into 3 regions on the Circos map (1: 1-192, 2: 198-296, 3: 313-457, 4: 546-567). In the N-terminal region 1, predicted intramolecular co-evolution is high, mainly in the structured segments, and that to the N-terminal side of α 3. Most of the predicted SMP structure in Mdm34 resides here and therefore, as for Mmm1, residues in the SMP domain predicted to be constrained and are therefore highly co-evolved. Regions 2, 3 and 4 are likely unstructured and within them, sites of intramolecular co-evolution are dispersed. Also, there is evidence for co-evolution between residues in the C-terminus (605-615) and multiple sites in region 1, interactions between the SMP domain and the end of the protein.

Regarding intermolecular co-evolution, Mdm34 potentially co-evolved with Mmm1, Mdm10, Pex11 and Lam6, through the structured part of the protein. In line with a recent model

(AhYoung et al. 2015), Mdm34 interacts with Mmm1 and Mdm12 for the transport of lipid. In the current study, co-evolution among the SMP domains of Mdm34, Mmm1 and Mdm12 is predicted. In particular, the regions flanking $\alpha 3$ of Mdm34 show the strongest evidence of co-evolution with Mmm1 and thus, this region may be of interest for understanding Mmm1-Mdm34 interactions. MI shared by the SMP domain of Mdm34 and both Pex11 and Lam6 was detected (supplementary figure S2C). Thus, the SMP domain and its adjacent sequence of Mdm34 may play an important role in the interactions with other proteins.

Mdm10

Initial phylogenetic analysis revealed two groups of Mdm10 proteins that were described as Mdm10 and Mdm10-like (Bay et al. 2012). In that study, four Mdm10-like proteins sequences were identified that were shorter than Mdm10 sequences and lacked the β -signals required for assembly into the MOM. Using the larger set of Mdm10 proteins currently available, it is clear that the Mdm10-like proteins are not phylogenetically distinct from those designated as Mdm10 (supplementary figure S3). Thus, the single group of Mdm10-related proteins was analyzed herein.

Mdm10 is a β -barrel-forming protein that part of the Tom40/VDAC superfamily (Bay et al. 2012; Flinner et al. 2013). The C-terminal part of the protein is predicted to fold into 14 β -strands (Fig. 2C, D), which can be superimposed onto the 19- β -stranded structure of mouse VDAC1 (Phyre2, PDB:3EMN). Multiple sequence alignments support a 19- β -stranded structure for Mdm10 (Bay et al. 2012; Flinner et al. 2013), but structural alignment of the first five β -strands with those of mouse VDAC1 was not supported (see supplementary table S3). Four α -helical regions are also predicted by the Phyre2 algorithm (Fig. 3D).

Based on MI, the reference set of Mdm10 sequences can be divided into four regions on the Circos map, (1: residues 419-480 and 1-108, 2:154-244, 3:277-339, 4:349-381). In this case, residues at the termini are considered together, as presumably β 19 and β 1 interact to close the barrel (see Bay et al. 2012; Flinner et al. 2013). A large subset of the intramolecular MI involves β -strands and may reflect assembly and stability of the barrel. Other sites of MI are in the regions of conserved residues that flank the α -helical segments of region 1, which may interact with other segments of the barrel.

Intermolecular co-evolution analysis predicts that a few residues in regions 1 and 4 of Mdm10 co-evolved with Mmm1, Pex11 and Lam6 (supplementary figure S2D). In region 1, these residues are present in the α -helix and in the segment predicted to be unstructured, while in region 4, β 14, β 15 and the intervening loop are involved. Interestingly, the most abundant interactions are between Mdm10 and Mmm1, which in most models of ERMES are not in direct contact. These sites of MI may suggest interactions between Mdm10 and Mmm1 that are independent of other ERMES proteins, or that form part of an extended network of interactions within the complex. Dispersed co-evolution was predicted between residues of Mdm10 and Lam6 and between Mdm10 and Pex11 (Fig. 3D and supplementary figure S2D), suggesting that these proteins may interact at the mitochondrial surface (Fig. 1).

Pex11

Peroxisomes have major roles in the scavenging peroxide and ROS and in β -oxidation of fatty acids (Mohanty et al. 2013). Pex11 is involved in peroxisome proliferation through its interactions with ERMES (Mattiuzzi Ušaj et al. 2015).

PRALINE analysis predicts that the Pex11 structure contains six α -helices and three TM

segments (Fig. 2E), two of which are embedded in longer regions of predicted α -helix (α 4 and α 5). There is no significant cluster of MI among different residues of Pex11 from the set of sequences used (Fig. 3E), which may suggest that regions of the protein do not interact with each other in a sequence-specific manner. Co-evolution analysis with CAPS2 demonstrated that individual residues of Pex11 potentially co-evolved with Mmm1, Mdm10, Mdm12, Mdm34, as described above, and Lam6 (supplementary figure S2E). In the α -helical domains α 1 and α 6, and adjacent to the C-terminal transmembrane domain, there are small clusters of residues that potentially interact with Mmm1, Mdm12 and Lam6. Another region in α 1 of Pex11 contains residues that may interact with the N-terminal segment of Mdm34, which is in agreement with physical interaction data (Mattiuzzi Ušaj et al. 2015). Dispersed sites of MI with Lam6 were detected along the full length of Pex11 (supplementary figure S2F), which agrees with the lack of physical evidence for strong Pex11-Lam6 interactions.

Lam6

Lam6 interacts with multiple complexes, ERMES, NVJ and vCLAMP (reviewed in Elbaz-Alon 2017). It is anchored to the ER membrane by a single transmembrane segment and is a member of StART (steroidogenic acute regulatory protein-related lipid transfer domain)-like/VAS_t (VAD1 Analog of StAR-related lipid transfer) domain-containing protein family (Daniele and Schiaffino 2016). The protein folds into seven α -helices, eleven β -strands and possesses one transmembrane domain, as predicted by PRALINE (Fig. 3F). Based on intramolecular MI, Lam6 residues can be divided into two regions (1: residues 254-370, and 2: 550-650) based on the reference set of sequences. Residues in region 2 fold into a StAR-like domain (Fig. 2E). Potential interactions were detected within and between these two regions,

particularly in β -strand-rich segments.

Recently, Tom70/71 of mitochondria were shown to be required for localization of Lam6 at the mitochondrial/ER junction (Murley et al. 2015). In addition to this interaction, extensive co-evolution of Lam6 with the ERMES components is suggested by intramolecular MI analysis. Groups of residues connected by MI were detected for Lam6 and Pex11, Mmm1, Mdm34 and Mdm10, with fewer interactions predicted with Mdm12 (supplementary figure S2F). Most of the potential co-evolution with other proteins is observed in the structured regions of Lam6.

Model for protein-protein interactions in ERMES and other complexes

The understanding of the interactions between eukaryotic intracellular membranes and the complexes supporting this network is continually expanding (see Elbaz-Alon 2017 for example). Purification of complexes, and identification of individual binding partners through pull-down experiments has revealed many intriguing details (supplementary table S1). Most of these interactions are supported by the MI analysis presented herein. Notably, most proteins have potential contacts with multiple members of ERMES (Fig. 1).

Mdm10 of the MOM displayed the least extensive groups of potentially co-evolving residues, mainly shared with Lam6 and Mmm1 (supplementary figure S2D). This protein is embedded in the MOM, and presumably few of its residues are available for protein-protein contacts. Tagged Mdm10 can be used to pull-down the other core ERMES subunits (Elbaz-Alon et al. 2015), indicating that its connection to the complex is strong. Lam6 was also co-purified in these experiments, supporting the co-evolution data. There was evidence for co-evolution among the SMP domains of Mmm1, Mdm12 and Mdm34 (Supplementary Fig. 2A,B,C). These may reflect the structural and hydrophobic requirements for lipid transport and for assembly of the

transport structures. The SMP domains of Mmm1 and Mdm12 likely collaborate in lipid transfer through a tetrameric structure and therefore contacts between the two proteins are expected. Interactions between Mdm34 and Mdm12 in a head-to-head organization are predicted (Jeong et al. 2016). In our experiments there was less evidence for co-evolution between Mdm34 and Mdm12, but this may be masked by the sequence and structural similarity shared by the two proteins that was noted by the authors.

One intriguing observation is the extensive MI shared between Lam6 and Pex11 (supplementary figure S2E,F). In Lam6, this region of MI overlaps with that shared by Lam6 and the ERMES components Mmm1, Mdm10 and Mdm34 (supplementary figure S2F). Lam6 is involved in regulation of the extent of mitochondrial contacts with ER, golgi and nuclear membranes (Elbaz-Alon et al. 2015). One hypothesis is that Pex11 may promote peroxisome-mitochondrial complexes by interacting with Lam6, thereby disrupting its interactions with parts of ERMES linked to mitochondria (Fig. 1). The MI shared between the N-terminus of Mdm12 and both Pex11 and Lam6 suggest that this region of the protein may be involved in these dynamic interactions, as it would be free from the SMP domain interactions.

Concluding remarks

In conclusion, our results support the topology of the ERMES complex as proposed recently (AhYoung et al. 2015, see Fig. 1) and reinforces the concept that to transport lipid, interactions between SMP domains of Mmm1, Mdm12 and Mdm34 are important. This study also predicted co-evolution among residues within SMP domains, identifying regions of potential importance for the structure of these domains. Our data also revealed clusters of residues that co-evolve in multiple ERMES proteins, suggesting interfaces in the complex formed by multiple

proteins, or multiple sub-complexes. The roles of Pex11 in peroxisome biogenesis and peroxisome-mitochondria interactions are beginning to be understood, and the potential interactions among Pex11, Lam6 and ERMES proteins may shed light on their roles in stabilizing the mitochondria-peroxisome contact sites, or in the function of ERMES in these contacts.

In future, understanding the interactions between Pex11, Lam6 and the ERMES proteins may help to elucidate the dynamic interactions and cross-talk among membrane contact sites, and data regarding co-evolution among the core ERMES proteins may provide direction to mutational studies used to interrogate the organization and functions of the ERMES complex.

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529 **Figure Legends**

530 **Figure 1.** Schematic diagram of ERMES complex and interactions. The model is based on those
531 of (Jeong et al. 2016) and (AhYoung et al. 2017) and the data described herein. The extra-
532 membrane barrels indicate SMP domains of the indicated proteins (1 in blue, Mmm1; 12 in
533 yellow, Mdm12; 34 in green, Mdm34), and the regions predicted to be unstructured are shown as
534 “clouds”. Mdm10 (grey barrel 10) is a β -barrel protein in the MOM. The transmembrane
535 domains of Mmm1 are shown as blocks in the ER, and the spacer between the transmembrane
536 and SMP domains is represented as a line. The complex is sketched as a dimer, as supported by
537 the heterotetrameric structure of Mmm1/Mdm12 (AhYoung et al. 2015). Pex11 (orange oval) and
538 Lam6 (purple crescent) are shown interacting with only one half of the dimer. A model for
539 interactions involving Lam6 are indicated on the right panel, and interactions with Pex11 that
540 might replace those involving Lam6 and therefore destabilize the complex are indicated to the
541 left. The transmembrane domain of Pex11, which anchors it to the peroxisomal membrane, is not
542 shown for simplicity. Potential interactions between Mmm1 and Mdm34, and between Mmm1
543 and Mdm10 are not shown, but could be envisioned as resulting from the three-dimensional
544 arrangement of the complexes. Note that Tom70/71 are involved in interactions with Lam6
545 (Murley et al. 2015), but were omitted for clarity.

546

547 **Figure 2.** Structural predictions. Panels **A)** and **B)** show a cartoon representation of the
548 superimposed predicted structures SMP domains obtained from Phyre2 for Mmm1 (blue),
549 Mdm12 (red) and Mdm34 (yellow). The fold predicted by Phyre2 for Mdm10 is shown in **C)**
550 and **D)**; note that the first 5 β -strands predicted by secondary structure analysis (for example see

(Bay et al. 2012) were not included in the model generated by alignment with the VDAC structure (PDB 3EMN). **E)** Fold predictions for Lam6. See supplementary table S3 for details regarding the quality of the fold predictions and alignment data.

Figure 3. Summary of MI and fold analysis of ERMES and its related proteins: **A)** Mmm1, **B)**, Mdm12, **C)** Mdm34, **D)** Mdm10, **E)** Pex11 and **F)** Lam6. An alignment was generated from untrimmed sequences using PRALINE (Simossis and Heringa 2005) and was analyzed for MI using MISTIC (Simonetti et al. 2013). The *N. crassa* sequence served as the reference and is presented in the figure; amino acid residues are indicated with single-letter code and position number in the untrimmed sequence. Red dots and larger numbers mark every 50th residue to aid in navigation. Residues linked by MI scores greater than 6.5 are considered statistically significant, and are connected by lines. Red lines indicate that the MI score for the two residues was in the top 5% of significant scores for that particular analysis. Similarly, black and grey lines connect pairs of positions with the top 5-30% of scores and the lower 70% of scores (Buslje et al. 2010) (Simonetti et al. 2013). The coloured rectangles indicate the conservation of residues across the MSA, with red indicating the highest and cyan the lowest levels of conservation. Proximity Mutual Information (pMI), describing networks of close residues with high MI are indicated by the black bars in the histogram inside of the conservation data. The sites of MI were divided into linear regions to aid in discussion; regions are indicated by circled numbers in medium-thickness lines in black (region 1), orange (region 2), yellow (region 3) and cyan (region 4). Secondary structure and transmembrane domains were predicted using the PSIPRED (Jones 1999) and PHOBIUS (Käll et al. 2004) algorithms, respectively, in the PRALINE package (Simossis and Heringa 2005). Regions of secondary structure predicted in all sequences in the

MSA are shown in red (α -helix), blue (β -strand) and green (transmembrane helices, TM). For discussion, these are numbered in boxes; the TM domain is not numbered to maintain the numbering for structural elements in SMP domains. For very short predicted regions of α -helix or β -strand, the α or β symbol was omitted and the number used alone for reasons of space. Regions rich in groups of residues coevolving with other proteins (see supplementary figure S2) are indicated by grey bars along the outermost black circle. The coloured numbers adjacent to these bars indicate the other proteins involved in this MI (1, Mmm1; 10, Mdm10; 12, Mdm12; 34, Mdm34; 6, Lam6; 11, Pex11). Regions with predicted structures aligning with known folds are indicated by the magenta arc and the grey regions within indicate residues that were omitted from the model structural model generated by Phyre2. Note that for some sequences, structural predictions generated via PRALINE do not correspond structures predicted from alignment with known protein folds in the database used by the Phyre2 algorithm.

Supplementary table S1. Summary of experimentally detected interactions among ERMES complex proteins.

Protein(s)	Type of Tag ¹	Protein(s) that interact	Method of isolating protein complex	Reference
Mmm1	Myc	Mdm10	Co-immunoprecipitation	(Boldogh et al. 2003)
Mdm12		Mdm10		
Mdm12		Mmm1		
Mdm34	Myc	Mmm1	Co-immunoprecipitation	(Youngman et al. 2004)
Mmm1	Protein A	Mdm10, Mdm12, Mdm34	IgG affinity chromatography	(Stroud et al. 2011)
Mmm1		Gem1		
Gem1		Mmm1, Mdm10, Mdm12, Mdm34		
Mmm1	Protein A	Mdm12, Mdm34, Mdm10 Δ L3(deletion in 84-136), Mdm10 Δ L15 (317-411), Mdm10 Δ L18 (452-481)	Affinity purification	(Flinner et al. 2013)
Mmm1	HA	Mmm1(Myc)	Co-immunoprecipitation	(Wideman et al. 2013)
Mmm1 (C-terminal)	TAP	Mdm10, mdm12, Mdm34, Gem1	Affinity purification	(Kornmann et al. 2011)
Mdm34 (C-terminal)		Mmm1, Mdm10, mdm12, Gem1		

MBP- Mmm1Δ1 (residues 122-426), MBP-Mmm1Δ5 (162-426), MBP-Mmm1Δ7 (182-426)	His	Mdm12	Affinity purification	(AhYoung et al. 2015)
Mdm34		Mdm12, complex of Mdm12/Mmm1Δ5		
Pex11	N- terminal part of Venus	C-terminal part of Venus Mdm34	Bimolecular fluorescence complementation (BiFC) assay	(Mattiuzzi Ušaj et al. 2015)
Mdm34	C- terminal fragment of ubiquitin	N-terminal fragment of ubiquitin Fused with Pex11	Membrane yeast two- hybrid (MYTH) assay	
Mmm1	HA	Mdm10, Mdm12, Mdm34, Gem1, Lam6	Pull down assay using beads against HA	(Elbaz-Alon et al. 2015)
Mdm10		Mmm1, Mdm12, Mdm34, Gem1, Lam6		
Mdm12		Mmm1, Mdm10, Mdm34, Gem1, Lam6		
Mdm34		Mmm1, Mdm10, Mdm12, Gem1, Lam6		
Lam6	GFP	Mmm1, Mdm10, Mdm34	Pull down assay using beads against GFP	

¹tags: HA, hemagglutinin; GFP, green fluorescent protein; TAP, tandem affinity purification;

References for Supplementary Table 1

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Supplementary table S2. Summary of sources of protein sequences used in the current study.								
		Trembl or Swiss Prot						
Species	File prefix/strain number	Mmm1	Mdm10	Mdm12	Mdm31	Mdm34	Lam6	Pex11
<i>Neurospora crassa</i>	aaNcrassa74A	A0A0B0E214	A0A0B0E367	A0A0B0EF10	Q7S930	A0A0B0E9A9	V5IMN0	V5IN16
<i>Ajellomyces capsulatus</i>	AJcapsulatusG186AR	C0NZU3	C0NFC2	C0NF00	C0P145	C0NY51	C0NDL0	C0NIA4
<i>Ajellomyces capsulatus</i>	AJcapsulatusNam1	A6RF31	A6QYP4	A6QYC8	A6R391	A6QVD2	A6QZS0	A6R9N9
<i>Ajellomyces dermatidis</i>	AJdermatitidisER3	C5GK63	C5GL32	C5GK63	C5GG53	C5GNU1	C5GUT9	C5GTQ6
<i>Ajellomyces dermatidis</i>	AJdermatitidisSLH14081	C5JYV0	C5K0G3	C5K0S2	C5JGU1	C5JVU4	C5JNE9	C5JPB1
<i>Arthroderma otae</i>	ARotae4605	C5FUT6	C5FMP1	C5FUT6	C5FY53	C5FRB0	C5FW54	C5FCQ2
<i>Ashbya gossypii</i>	ASHgossypii10895	Q75CC2	M9N4C2	Q75CC2		Q755S6	Q75CZ1	Q74Z07
<i>Aspergillus clavatus</i>	ASPclavatus1007	A1CM86	A1CBT0	A1CNY1	A1CE31	A1CHU1	A1CA42	A1CT62
<i>Aspergillus flavus</i>	ASPflavus200026	B8NDR8	B8NM08	B8MZJ8	B8N2L2	B8N0Q7	B8NDZ6	B8N2F6
<i>Aspergillus niger</i>	ASPniger51388	A2R4N0	A2R3N6	A2QAU8	G3YEM9	A2QJH4	A2R969	A2QVT6
<i>Aspergillus oryzae</i>	ASPoryzae42149	Q2U9A7	A0A064BG38	A0A064AZV3	I7ZM30	Q2UJF5	Q2U8W2	Q2UM26
<i>Aspergillus terreus</i>	ASPterreus	Q0CZL5	Q0CJH2	Q0CZL5	Q0CFX2	Q0CZY7	Q0CAG7	Q0CK93
<i>Botryotinia fuckeliana</i>	BfuckelianaBcDW1	G2YJ50	G2XNN4	A6RLP9	G2YTA1	M7UTA8	M7U4B0	M7URU9
<i>Candida albicans</i>	CAalbicansSC5314	A0A0A6LXE0	Q59LL4	A0A0A6M5S4	Q5AI66	A0A0A6MTH0	Q59XR6	Q59QX0
<i>Candida albicans</i>	CAalbicansWO1	C4YI11	C4YIG3	C4YTH8	C4YEX9	C4YE34	C4YDS7	C4YS89
<i>Candida dubliniensis</i>	CAdubliniensisCD36	B9WFC9	B9WBE5	B9WK07	B9W777	B9W824	B9W8D1	B9WJA8
<i>Candida glabrata</i>	CAGlabrata2001	Q6FVT7	Q6FWV0	Q6FVF2	Q6FMB2	Q6FQE0	Q6FUI2	Q6FW65
<i>Candida tropicalis</i>	CAtropicalisMYA3404	C5M1Z6	C5M6Z4	C5MFK7	C5ME38	C5MFK7	C5M978	C5M8N4

<i>Chaetomium globosum</i>	CHglobosum6205	Q2HA83	Q2H740	Q2HDE7	Q2GZY0	Q2H9Y1	Q2H2V5	Q2GSX3
<i>Clavispora lusitanae</i>	CLlusitanae42720	C4Y0Q6	C4XVQ6	C4YBT3	C4Y8D1	C4Y6F4	C4XZU5	C4YB30
<i>Coccidioides immitis</i>	COCimmitisRS	Q1E432	A0A0D6K9M5	Q1E2F1	A0A0J8UMF9	Q1E4T3	J3K7F5	A0A0J8RIB0
<i>Coprinopsis cinerea</i>	COPcinerea130	A8N1W8	A8NWS6	A8NEF5	A8N5C0	A8NYS9	A8N3J2	A8N1T3
<i>Cryptococcus neoformans</i> var. <i>neoformans</i> serotype D	Crneoformans B3501A	P0CO81	P0CO67	P0CO69	F5HCT4	P0CO71	Q55Z77	Q560N5
<i>Cryptococcus neoformans</i> var. <i>neoformans</i> serotype D	CRneoformans JEC21	P0CO80	P0CO66	P0CO68	Q5K9K2	P0CO70	Q5KNJ2	Q5KPN1
<i>Debaromyces hansenii</i>	Dhansenii36239	B5RUL6	Q6BTX9	B5RUL6	Q6BJ74	Q6BUS9	Q6BLZ1	Q6BYZ1
<i>Emericella nidulans</i>	EnidulansA4	Q5AQA2	Q8J0L4	Q5BF59	Q5B0Z4	Q5BBM5	Q5BB65	Q5BC09
<i>Kluveromyces lactis</i>	KLlactis8585	Q6CUC3	Q6CKU5	Q6CUC3	Q6CUA4	Q6CYC1	Q6CRY1	Q6CPV7
<i>Komagataella pastoris</i>	KOpastorisGS115	C4R892	C4QWJ4	C4R415	C4R1E7	F2QPU4	C4QW28	C4R0W1
<i>Laccaria bicolor</i>	LACCbicolorS238N	B0DQ09	B0DK33	B0DQ09	B0D138	B0CXH5	B0CNP4	B0CQI0
<i>Lachancea thermotolerans</i>	LACHthermotolerans 56	C5DCI0	C5DG70	C5DEN8	C5DFT6	C5DLL1	C5DNQ6	C5DJX1
<i>Lodderomyces elongisporus</i>	LOelongosporus 11503	A5E3T2	A5DUG6	A5E771	A5DVW7	A5DUL5	A5DU13	A5E5J3
<i>Magnaporthe oryzae</i>	MAGoryzae7015	A4RM19	L7IKM1	L7IG02	L7J6G4	L7I6Z8	G4MZL9	G4MVK9
<i>Meyerozyma guilliermondii</i>	MEguilliermondii6260	A5DGW8	A5DCL4	A5DAR4	A5DI60	A5DLK4	A5DIL5	A5DJQ2
<i>Neosartorya fischeri</i>	NEfischeri1020	A1DLT0	A1DDI7	A1D1T8	A1DM40	A1D2P6	A1DFB7	A1DMH4

<i>Neosartorya fumigata</i>	NEfumigataCEA10	B0YD09	B0Y240	B0XN24	Q4WNG8	B0Y6I4	B0Y3L7	B0Y7G2
<i>Paracoccidioides brasiliensis</i>	PAbrasiliensisPb03	C0SGL5	C0SE00	C0SE33	C0S1B0	C0S713	C0SHH9	C0SIL4
<i>Paracoccidioides brasiliensis</i>	PAbrasiliensisPb18	C1GHQ8	C1GI10	C1GHQ8	C1G9N3	C1G520	C1G613	C1GMM4
<i>Paracoccidioides lutzii</i>	PAlutziiMYA826	C1GXJ4	C1GVA2	C1H3V1	C1GQR9	C1HBX6	C1H1U5	C1HBR8
<i>Penicillium chrysogenum</i>	PEcyrsogenum28089	B6HDM7	B6HL48	B6HAR6	A0A161ZDK6	B6HR25	B6HVA6	A0A167VSS4
<i>Saccharomyces cerevisiae</i>	SAcerevisiaeR103	A0A024Y027	A0A024Y807	A0A024XVW0	A0A024XWQ4	A0A024 Y6Q4	A0A024XZW4	A0A024XYH0
<i>Scheffersomyces stipitis</i>	SCHEstipitis58785	A3LSW7	A3LT10	A3LWH1	A3GIA0	A3GFQ7	A3LUW6	A3LTB9
<i>Schizosaccharomyces japonicus</i>	SCHljaponicusyFS275	B6K6N3	B6K463	B6K4Z5	B6K443	B6K0B1	B6K0D6	B6K617
<i>Schizosaccharomyces pombe</i>	SCHlpombe972	O74368	O13814	A0A0B7F7C1	Q10070	Q9UUC9	O42976	Q10333
<i>Sclerotinia sclerotiorum</i>	SCLsclerotiorum18683	A7ELE2	A7F1G7	A7ELE2	A7EG50	A7EWF5	A7EB90	A7EAS7
<i>Uncinocarpus reesii</i>	UNreesii1704	C4JP24	C4JV00	C4JY59	C4K080	C4JQ45	C4JN45	C4JGK9
<i>Ustilago maydis</i>	USmaydis521	A0A0D1CN66	A0A0D1CER6	A0A0D1E2J7	A0A0D1E708	A0A0D1E6T6	A0A0D1CQA5	A0A0D1DZ09
<i>Vanderwaltozyma polyspora</i>	Vpolyspora22028	A7TFL9	A7TNL0	A7TFP8	A7THL9	A7TES0	A7TFN7	A7TQZ8
<i>Yarrowia lipolytica</i>	Ylipolytica122	Q6CAP9	Q6CAG4	Q6CI13	Q6C7F9	Q6C7W0	Q6CGT7	Q6CD37
<i>Zygosaccharomyces rouxii</i>	Zrouxii2623	C5DRQ1	C5E1U4	C5DQQ7	C5DW29	C5E4T8	C5DX62	C5DSW9

Supplementary table S3. Phyre² prediction data for proteins investigated in this study.

Protein name ^a	Top template and protein name	Confidence (%) & coverage (%)	Aligned residues and identity ^b
MDM10 (Q7SBE0)	C2k4tA; VDAC (voltage dependent anion channel)	98.5 & 42	174-480 (19%)
MDM12 (Q7SEZ6)	C4p42B; extended synaptotogmin-2	96.3 & 16	521-673 (18%)
MDM32/31 (Pod-1 ^d ; Q7S930)	D1pgl11; nucleoplasmin-like VP from ssRNA viruses	63.5 ^c & 7	341-429 (20%)
MDM34 (Q7RZK9)	C4p42B; extended synaptotogmin-2	99.3 & 23	7-182 (13%)
MMM1 (Q9P353)	C4p42B; extended synaptotogmin-2	100 & 43	116-334 (16%)
LAM6 (V5IN16)	C4tyzB star-related lipid transfer protein 6	99.6 & 11 97.7 & 32	268-370 (26%) 496-676 (13%)
PEX11 (V5IN16)	C2jwA; uncharacterized protein q5e7h1 from <i>Vibrio fischeri</i>	15.6 & 9	8-28 (29%)

^a protein predicted from *N. crassa* genome used for analysis; SwissProt number given in parentheses

^b identity in aligned region

^c low confidence

^d *Neurospora crassa* gene name

(<https://data.broadinstitute.org/annotation/genome/neurospora/MultiHome.html>)

Supplementary file S1: Phylogenetic Analysis

Mdm10 and Mdm31/32 sequences were extracted from online databases NCBI and JGI (MycoCosm) (<http://www.ncbi.nlm.nih.gov/>; Grigoriev et al., 2012). Sequences were extracted with blastp and compiled and aligned with PRALINE. Genedoc (Nicholas et al., 1997) was used for manual editing of multiple sequence alignments generated by PRALINE (Simossis and Heringa, 2005). The PRALINE program was also utilized to gain some preliminary secondary structure predictions by selecting the SSPRO option. The PRALINE alignments were edited with GeneDoc in order to remove ambiguously aligned regions and regions with gaps. These alignments formed the basis for phylogenetic and co-evolutionary analysis.

Evolutionary analyses were conducted in Molecular Evolutionary Genetics Analysis version 6 (MEGA6; Tamura et al. 2013). The evolutionary history for the protein data sets were inferred by using the Maximum Likelihood method (ML) based on the Le_Gascuel_2008 model (Le and Gascuel, 1993) for the mdm31/32 data set or the JTT (Jones-Taylor; Jones et al. 1992) model for the mdm10 data set. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 1.9163)]. All positions containing gaps and missing data were eliminated. The model for the ML analysis was chosen based on the output by the “best model” option within MEGA. The data sets were also analyzed with the Neighbor and Parsimony programs as implemented in MEGA. In all cases the bootstrap option (1000 pseudoreplicates) was implemented.

Phylogenies were also inferred by bayesian analysis carried out using the MrBayes program (v3.1; Ronquist and Huelsenbeck, 2003; Ronquist, 2004). Bayesian inference phylogenies were initiated from a random starting tree and four chains were run simultaneously for 3,000,000 generations (for the mdm10 alignment) or 5,000,000 generations (for the mdm31/32 data set) to a standard deviation of split frequencies less than 0.01. Models implemented in MrBayes were the same as those applied in the ML analysis performed by MEGA. The first 30 % of the generated trees were discarded and the remaining trees were used to compute the posterior probability values. Phylogenetic trees were drawn using the TREEVIEW (Page 1996) program using either the MEGA tree files or the Mr. Bayes tree files. Final trees reported in this report are based on the fifty percent majority rule consensus trees obtained from the Mr. Bayes analysis. Trees were annotated using Corel Draw (version 14; Ottawa, Canada).

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Supplementary figure S1. Phylogenetic analysis of Mdm31/32 proteins. The tree is based on sequences denoted Mdm31 or Mdm32 in various databases, which were analyzed as indicated in Supplementary File 1. Tree topology and node support are based on Bayesian analysis. Solid circles, squares and triangles represent supportive values (95–100%), (85–94%) and (50–84%) respectively, as obtained from a 50% majority Bayesian consensus tree. Nodes that received less than 50% support were collapsed. The branch lengths are proportional to the number of substitutions per site. Accession numbers are underlined and listed next to species names. The tree is rooted with the *Gonapodya prolifera* sequence. Numbered nodes are discussed in the accompanying text.

Draft

A

Mmm1

F

Supplementary figure S2. Summary of MI for pairs of ERMES proteins. Pairs of trimmed multiple sequence alignments (MSA) for the indicated protein and other members of ERMES/Pex11/Lam6 were analyzed via MISTIC (Simonetti et al. 2013). Intramolecular MI for each protein is shown on a Circos representation (A) Mmm1, B) Mdm12, C) Mdm34, D) Mdm10, E) Pex11 F) Lam6. Lines connect pairs of positions with MI greater than 6.5 (Buslje et al. 2010). Red lines indicate the top 5% of significant MI scores, black lines the top 30% and grey lines the remaining 70%. For pairwise analysis, the groups of residues sharing MI with other ERMES members are aligned along the black circle and are indicated for the pairs with Mdm10 (red numbers), Mdm12 (green), and Mdm34 (blue). Residues potentially co-evolving with Pex11 (brown numbers) and Lam6 (purple) are indicated on brown and purple circles, respectively. The thick magenta arc indicates the region modeled through Phyre2 (Kelley et al. 2015) to form a tertiary structure similar to that contained in the Phyre2 fold library (see Supplementary Table 3). The grey region in the arc indicates residues that were omitted from the model.

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Weblogo for the C-terminal segment of the Mdm10 alignment

beta signal

Supplementary figure S3. Phylogenetic analysis of Mdm10 proteins. The tree in the upper panel is based on 150 sequences denoted Mdm10 or Mdm10-like in various databases and which were analyzed as indicated in Supplementary File 1. Tree topology and node support are based on Bayesian analysis. Solid circles, squares and triangles represent supportive values (95–100%), (85–94%) and (50–84%) respectively, as obtained from a 50% majority Bayesian consensus tree. Nodes that received less than 50% support were collapsed. The branch lengths are proportional to the number of substitutions per site. Accession numbers are underlined and listed next to species names. The tree is rooted with the *Batrachochytrium dendrobatidis* sequence. Numbered nodes are discussed in the accompanying text.

The lower panel presents the WebLogo (Crooks et al. 2004) for the C-terminal 18 amino-acid segment of the Mdm10 alignment. This segment encompasses the β -signal as described in (Imai et al. 2011) required for assembly of β -barrel proteins into the MOM.