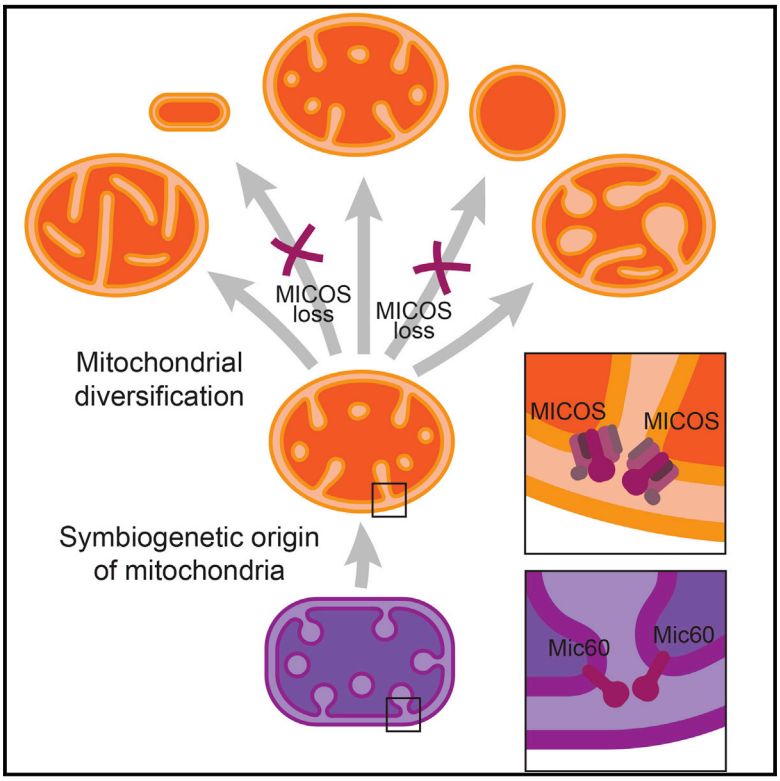
Report

Current Biology

Ancient Homology of the Mitochondrial Contact Site and Cristae Organizing System Points to an Endosymbiotic Origin of Mitochondrial Cristae

Graphical Abstract



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In Brief

Muñoz-Gómez et al. identify a prokaryotic homolog of eukaryotic Mic60 (a MICOS component that is required for cristae morphogenesis), thus providing evidence for the endosymbiotic origins of mitochondrial cristae. The ubiquity of MICOS implies a general eukaryotic mechanism for mitochondrial cristae formation and maintenance.

Highlights

- MICOS is an ancient and widespread eukaryotic protein complex
- Eukaryotes that have lost MICOS lack mitochondrial cristae
- MICOS has an endosymbiotic origin from α-proteobacteria

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Ancient Homology of the Mitochondrial Contact Site and Cristae Organizing System Points to an Endosymbiotic Origin of Mitochondrial Cristae

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SUMMARY

Mitochondria are eukaryotic organelles that originated from an endosymbiotic α-proteobacterium. As an adaptation to maximize ATP production through oxidative phosphorylation, mitochondria contain inner membrane invaginations called cristae. Recent work has characterized a multi-protein complex in yeast and animal mitochondria called MICOS (mitochondrial contact site and cristae organizing system), responsible for the determination and maintenance of cristae [1–4]. However, the origin and evolution of these characteristic mitochondrial features remain obscure. We therefore conducted a comprehensive search for MICOS components across the major groups that encompass eukaryotic diversity to determine the extent of conservation of this complex. We detected homologs for the majority of MICOS components among opisthokonts (the group containing animals and fungi), but only Mic60 and Mic10 were consistently identified outside this group. The conservation of Mic60 and Mic10 in eukaryotes is consistent with their central role in MICOS function [5–7], indicating that the basic mechanism for cristae determination arose early in evolution and has remained relatively unchanged. We found that eukaryotes with ultrastructurally simplified anaerobic mitochondria that lack cristae have also lost MICOS. We then searched for a prokaryotic MICOS and identified a homolog of Mic60 present only in α-proteobacteria, providing evidence for the endosymbiotic origin of mitochondrial cristae. Our study clarifies the origins of mitochondrial cristae and their subsequent evolutionary history, provides evidence for a general mechanism of cristae formation and maintenance in eukaryotes, and points to a new potential factor involved in membrane differentiation in prokaryotes.

RESULTS AND DISCUSSION

MICOS Is an Ancient Eukaryotic Protein Complex that Co-occurs with Mitochondrial Cristae

Due to their α-proteobacterial origins, mitochondria are double membrane-bound organelles consisting of four major compartments (Figure 1): the mitochondrial inner membrane (MIM), the mitochondrial outer membrane (MOM), the intermembrane space (IMS), and the mitochondrial matrix. The MIM can be further divided into two functionally and compositionally distinct domains: the inner boundary membrane (IBM) and the cristae membrane (CM). The IBM is closely apposed to the MOM and appears to be predominantly involved in protein translocation and solute transport [9], whereas the CM is the site of oxidative phosphorylation and is formed by MIM invaginations that protrude from crista junctions (CJs) into the mitochondrial matrix [10]. The mitochondrial contact site and cristae organizing system (MICOS) is a multi-protein complex, comprising Mic10, Mic12, Mic19, Mic25, Mic26, Mic27, and Mic60, that localizes to CJs (Figure 1) and whose disruption leads to virtual CJ loss, altered mitochondrial ultrastructure, and impaired respiratory function [1–4]. Thus, a primary function of MICOS has been proposed to be the formation of CJs and consequently the formation and maintenance of mitochondrial cristae [5–7, 11, 12]. MICOS subunits have been reported in *Saccharomyces cerevisiae* [1, 3], *Caenorhabditis elegans* [13], and *Homo sapiens* [14–16]; however, the extent to which our knowledge about MICOS function might be applied to diverse eukaryotes is unknown. We therefore investigated the phylogenetic distribution of MICOS subunits across the tree of life in order to shed light on the evolutionary history of MICOS and the origin and evolution of mitochondrial cristae.

Using a combination of BLAST and hidden Markov model (HMM) homology searching algorithms, we searched for the homologs of MICOS subunits in a representative subset of genomes and transcriptomes from the major groups that

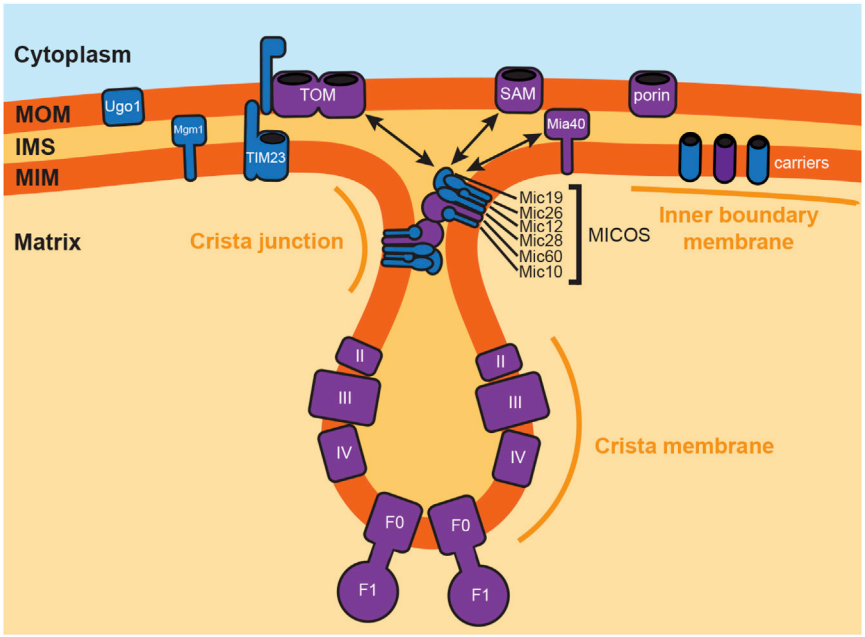

Figure 1. MICOS Is Involved in CJ Formation and Determination of Mitochondrial Cristae

MICOS is composed of six subunits in *S. cerevisiae* [4], two core subunits, Mic60 and Mic10, and the additional subunits Mic19, Mic26, Aim37 (Mic28), and Mic12. MICOS localizes to CJs and ensures that the structurally and functionally different cristae and IBMs remain connected [1–5]. Additionally, MICOS interacts with several proteins at contact sites of the mitochondrial envelope, including TOM and SAM in the MOM and Mia40 in the IMS [1, 2, 8].

encompass eukaryotic diversity (see Supplemental Experimental Procedures). Our methods allowed us to identify putative MICOS components from every major eukaryotic lineage (Figures 2 and S1A).

Mic60 is the central and largest protein in MICOS, and together with Mic10, constitutes the core of the complex [1, 5, 7, 18]. Loss of either protein leads to the most severe phenotypes, relative to other components. Mutant *S. cerevisiae* or human mitochondria lacking Mic60 or Mic10 lose virtually all CJs and accumulate stacked internal membranes in their matrix with nearly no visible connections to the IBM [1, 6]. We were able to identify both Mic10 and Mic60 in every major lineage of eukaryotes including fungi, animals, amoebozoans, excavates, SAR, and archaeplastids, among others (Figures 2 and S1A). This finding is in agreement with their importance in opisthokont mitochondria and suggests that these proteins also play a central role in MICOS function in diverse eukaryotes. Since MICOS constitutes the molecular basis of CJs, our analyses suggest that CJs are universally present in mitochondria even though CJs have so far only been observed in animals [19], fungi [20], and amoebae [21]. The conservation of MICOS indicates that a general mechanism of cristae formation and maintenance through MICOS action at CJs can be applied to all eukaryotes that exhibit mitochondrial cristae.

Further support for the fundamental role of MICOS in cristae formation and maintenance emerges from our observation that taxa that lack MICOS exhibit ultrastructurally simplified mitochondria without cristae (e.g., mitosomes and hydrogenosomes). Species lacking MICOS include the fungus *Piromyces* sp., the microsporidians *Encephalitozoon cuniculi* and *Nosema ceranae*, the amoeba *Entamoeba histolytica*, and the metamonads *Giardia intestinalis* and *Trichomonas vaginalis* (Figures 2 and S1A). These organisms represent diverse branches on the tree of eukaryotes that have independently adapted to low-oxygen environments during evolution [22]. Ultrastructural simplification through cristae loss has accompanied the irreversible loss of the respiratory chain and the capability to perform chemiosmotic ATP production [22]. Thus, the loss of the MICOS complex could be interpreted as the decisive step in the path toward complete loss of the characteristic internal cristate structure of aerobic mitochondria.

In support of this claim, we have identified MICOS components in organisms that bear substantially reduced anaerobic mitochondria but retain some inner membrane ultrastructure. For example, *Blastocystis hominis* possesses cristae in its anaerobic mitochondrion [23] and encodes the full core complement of MICOS subunits in its genome. Furthermore, an extremely divergent Mic10 ortholog was identified in *Cryptosporidium muris*, which retains tubular cristae, as well as a Krebs cycle, an incomplete respiratory chain (i.e., complex I and II), and an alternative oxidase (AOX) [24, 25]. Its close relative *Cryptosporidium parvum* also possesses a divergent Mic10, which might account for the abnormal inner-membrane folds present in its even more reduced mitosome [26]. These correlations further support the indispensable role of MICOS in cristae morphogenesis and maintenance.

Similar to Mic10 and Mic60, Mic19 also appears to exhibit a wide, although less regular, distribution among eukaryotes (Figure 2). In this case, the short nature and lower sequence conservation of Mic19 (containing either DUF737 or DUF1690) hinders a confident assessment of homology in lineages outside the opisthokonts (Figures 2 and S1A, gray circles). MICOS subunits Mic25, Mic26, Mic27, Mic12, and Aim37 have more restricted distributions (Figures 2 and S1A). Of these, Mic26 is found among opisthokonts, whereas Mic25 and Mic27 are restricted to vertebrates, and Mic12 is restricted to fungi. Aim37 is found exclusively in the Saccharomycetales (see below).

Two other proteins, OPA1 and MICS1, have been implicated in maintaining overall mitochondrial morphology and cristae integrity, as well as in regulating the apoptotic release of cytochrome c in mammalian cells [27, 28]. In order to determine whether these proteins could also be generally responsible for maintaining mitochondrial cristae across eukaryotic diversity, we searched our genome database for their orthologs. Our analyses demonstrate that Opa1 and a related fungal protein Mgm1 are restricted to holozoans and fungi, respectively (Figure S1B). In

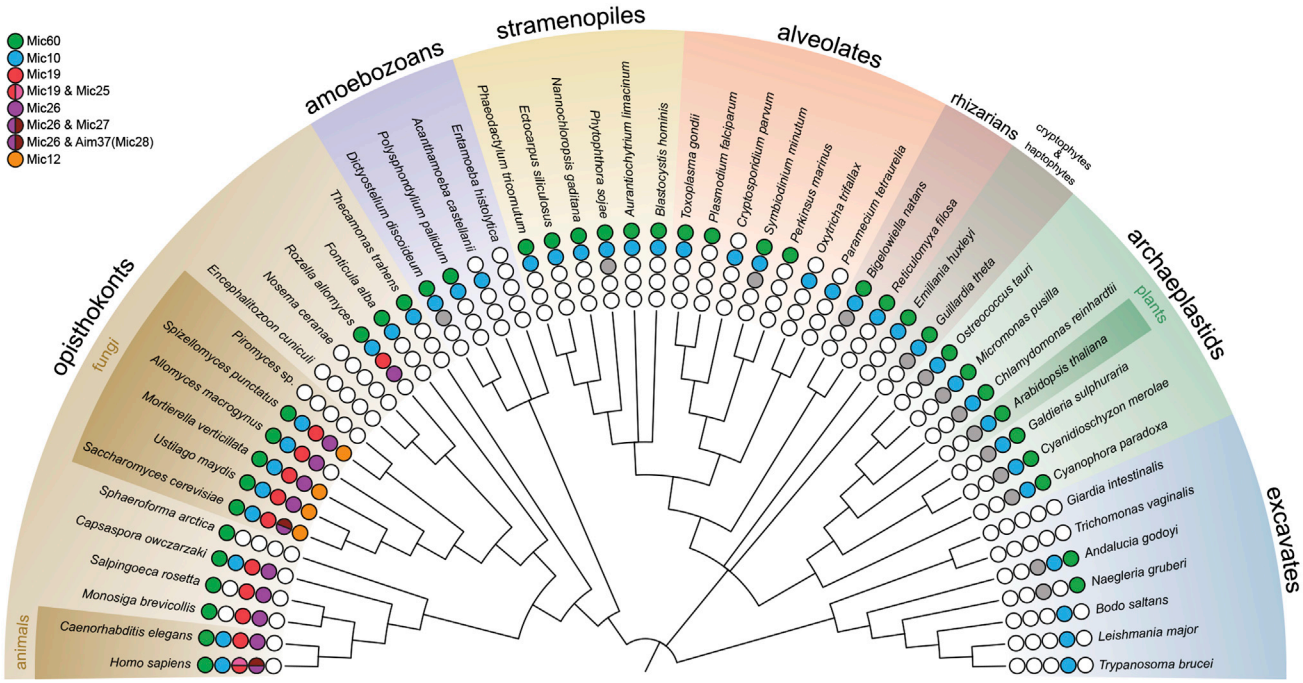

Figure 2. Distribution of MICOS Subunits across the Eukaryote Evolutionary Tree

Consensus evolutionary tree of eukaryotes [17]. Major eukaryote groups are represented by species for which whole-genome data are available. The classical multicellular lineages (animals, fungi, and plants) are highlighted within these major groups. MICOS subunits were identified by a combination of BLAST and HMM homology searching methods. Colored circles at tips indicate presence of MICOS subunits. Mic60, Mic10, Mic19, Mic25, Mic26, and Mic27/Aim37 (Mic28) are indicated by green, blue, red, red-pink, purple, and purple-brown circles, respectively. Gray circles indicate potential Mic19 orthologs in non-opisthokont lineages (see Figures S1A and S2 and Table S1).

contrast, MICS1 has a patchy distribution across eukaryotes, indicating an ancient origin, followed by repeated loss (Figure S1B). It has been suggested that Opa1 and Mgm1 are orthologs, although this has not been convincingly demonstrated [29]. Both proteins are closely related to dynamin, but our results suggest that each protein has independently evolved from its respective holozoan or fungal dynamin, rather than sharing a direct common ancestor. Further bioinformatic and phylogenetic analyses focused on this aspect of the dynamin superfamily must be conducted to fully understand the relationship between these two proteins.

Paralogy, Convergence, and Origin of MICOS Subunits Mic25, Mic27, and Aim37/Mic28

The distribution of Mic60, Mic10, and possibly Mic19, across eukaryotic diversity allows us to infer that the last eukaryote common ancestor (LECA) made use of a MICOS complex consisting of these three proteins. Other MICOS subunits were later acquired as groups diverged (e.g., Mic26 in opisthokonts and Mic12 in fungi). Vertebrate Mic19 and Mic25 contain similar CHCH domains [15]. Their restricted co-occurrence within the vertebrate lineage strongly suggests that Mic25 is a paralog of Mic19 that evolved by duplication and divergence from an ancestral Mic19 of wide eukaryotic distribution. Unfortunately, the CHCH domain is too short to be informative in rigorous phylogenetic analyses, and thus, the paralogy of Mic19 and Mic25 could not be confirmed.

Mic26, Mic27, and Aim37 are obviously related, constituting a protein family characterized by the presence of an ApoO domain [16]. It has therefore been suggested that Aim37 be renamed as fungal Mic27 to reflect this relationship [4]. In order to clarify the specific evolutionary relationships among the members of this gene family, we performed phylogenetic analyses of these ApoO domain-containing proteins (see Supplemental Experimental Procedures). We found that Aim37 is not orthologous to vertebrate Mic27 (Figure S2A). Instead, our analyses revealed that Mic27 and Aim37 are paralogs of Mic26 that originated from independent duplications in vertebrates and Saccharomycetales, respectively (Figures S2A and S2B). Hence, we suggest Aim37 be renamed Mic28, specific to the Saccharomycetales.

Similar to the evolutionary history of other macromolecular assemblies [30] (e.g., the nuclear pore complex and the spliceosome), MICOS has increased in complexity through the process of gene duplication followed by sequence divergence and retention of paralogs. This phenomenon is observed independently in vertebrates and the Saccharomycetales, in which Mic26 gave rise to its paralogs Mic27 and Aim37 (Mic28), respectively. This interpretation of the evolution of MICOS wholly relies upon the functionally identified and validated MICOS components in *S. cerevisiae*, *C. elegans*, and *H. sapiens*. Not only is it possible that independent MICOS expansions have occurred in other parts of the tree, but lineage-specific loss of hitherto undiscovered ancient MICOS components may have also occurred in the opisthokonts. Unfortunately, without functional data, it is

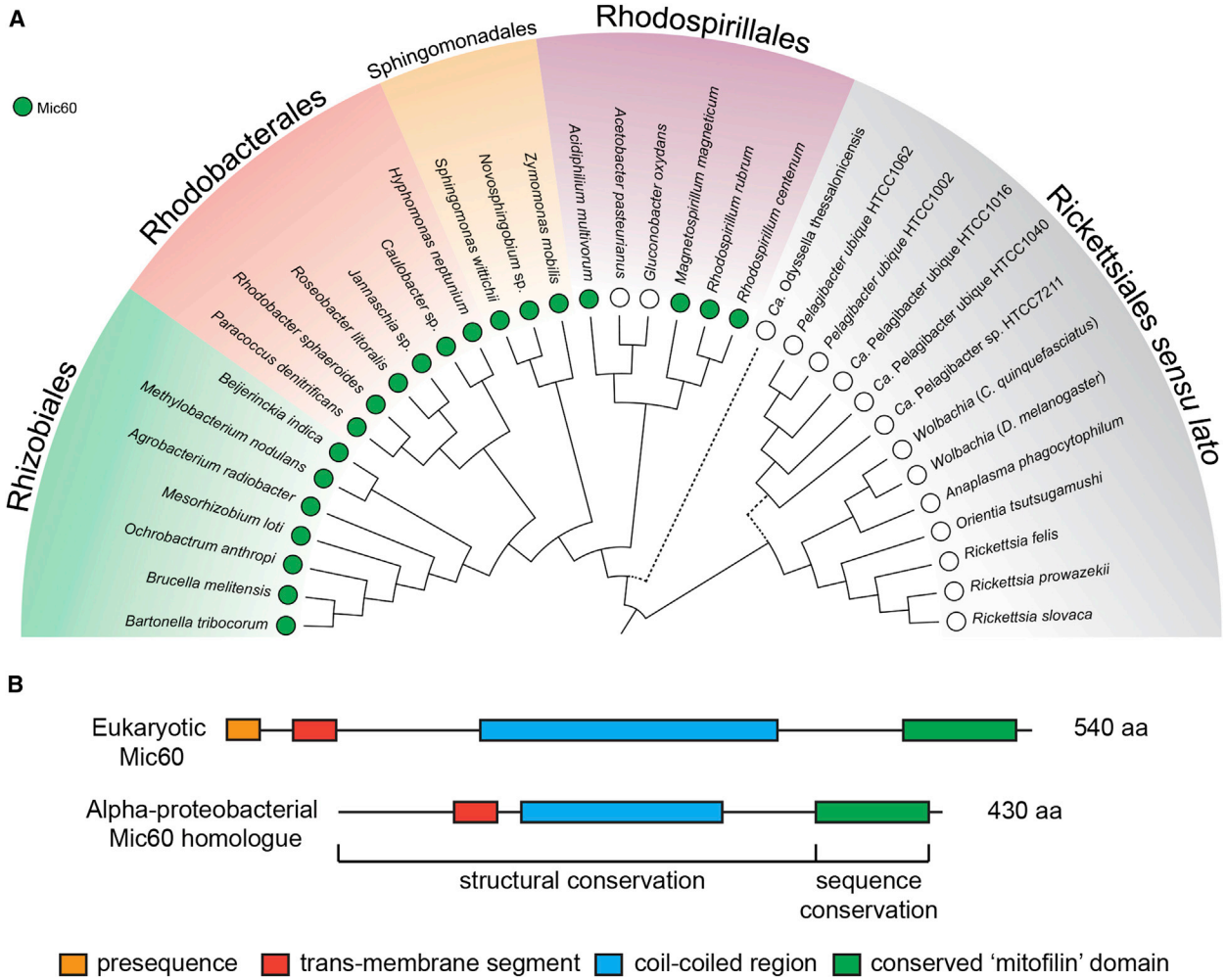

Figure 3. Distribution and Domain Architecture of α-Proteobacterial Mic60

(A) Distribution of MICOS (Mic60 homologs) in α-proteobacteria. Consensus phylogenetic tree of α-proteobacteria based on [31] containing a representative set of species from the major orders recognized in the group. α-proteobacterial homologs of Mic60 were identified by HMM searches.

(B) Conserved Mic60 protein domain architecture in eukaryotes and prokaryotes. Structural motif predictions were carried out using TMHMM, TMpred, and COILS (see Figure S3 and Table S2).

impossible to investigate this further. These possibilities further underscore the importance of establishing model systems that span the diversity of eukaryotes in order to gain insight into the evolution of cellular systems.

MICOS Core Subunit Mic60 Has α-Proteobacterial Origins

Our search for MICOS homologs in complete databases led to the identification of bacterial homologs of Mic60 (Figures 3A and S3). These homologs were restricted to α-proteobacteria, the bacterial progenitor lineage of mitochondria. Although Mic60 homologs are present in diverse members of the α-proteobacteria, they are absent from the SAR11, Anaplasmataceae, and Rickettsiaceae groups (''Rickettsiales'' sensu lato) (Figure 3A).

In order to assess the likelihood that α-proteobacterial Mic60 might retain a similar function to that of eukaryotic Mic60, we investigated structural features of the candidate proteins. Using HMMER3, we determined that, similar to their eukaryotic counterpart, α-proteobacterial Mic60 has a conserved C-terminal mitofilin domain readily retrieved using HMM searches. The rest of the protein is not well conserved at the sequence level, even among eukaryotes, but using TMHMM, TMpred, and COILS as bioinformatic predictors for secondary protein structure, we were able to reveal that prokaryotic Mic60 has an N-terminal transmembrane segment as well as a central coil-coiled region (Table S2). These structural motifs are found in corresponding positions to those of their eukaryotic homologs (Figure 3B). We conclude that the identified Mic60 homologs among α-proteobacteria retain the same overall structure as eukaryotic Mic60, providing additional support for its functional conservation. However, we cannot rule out the possibility that Mic60 in α-proteobacteria might be performing a different function and was only later recruited to stabilize cristae and improve mitochondria function via its propensity for homotypic interactions at the IMS [5, 18].

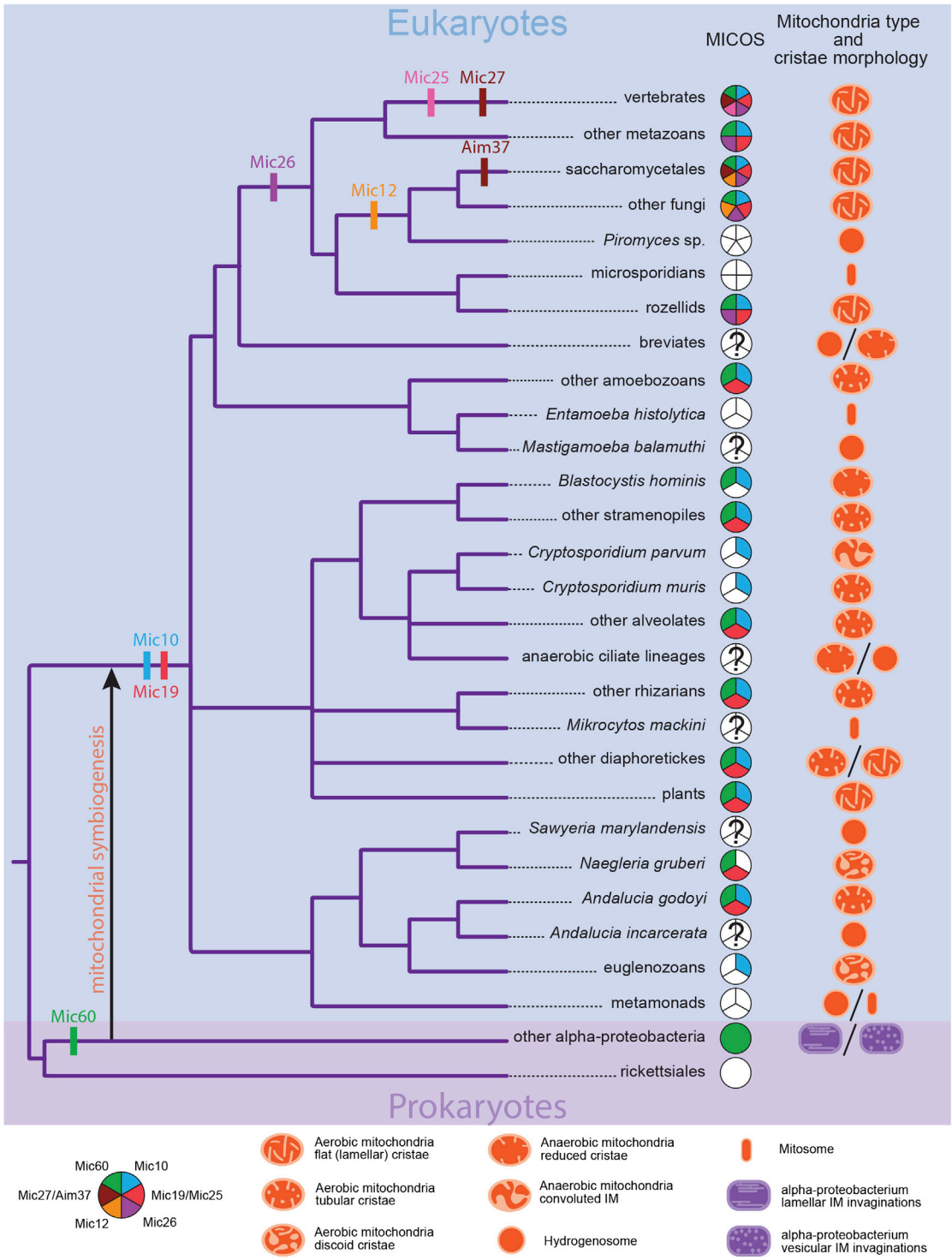

Figure 4. Evolutionary History of MICOS

MICOS has an endosymbiotic origin from within the α-proteobacteria. The core MICOS component Mic60 was acquired with the α-proteobacterial endosymbiont that gave rise to the mitochondrion. Later, Mic10 and Mic19 were added to the ancestral MICOS (Mic60) in the eukaryote stem lineage before the diversification of eukaryotes into modern groups. Other MICOS components (Mic26, Mic25, Mic27, Mic12, and Aim37 [Mic28]) were later added during opisthokont evolution (see Results and Discussion). Several eukaryotic groups (e.g., *Piromyces* sp., microsporidians, and metamonads) with simplified mitochondria (e.g., mitosomes and hydrogenosomes) lost MICOS and therefore cristae (white pies). Other anaerobic linages (e.g., anaerobic ciliates and breviates) that exhibit mitochondria with no cristae are hypothesized to have lost MICOS as well (white pies with question marks). Cristae morphotype in aerobic mitochondria is independent of MICOS subunit composition as seen from their lack of correlation across eukaryote diversity. Most α-proteobacteria, excluding rickettsiales, encode a Mic60 homolog in their genomes, which is in congruence with most of them having complex ICMs that invaginate from their cytoplasmic membranes.

We have been able to trace the evolutionary history of MICOS to α-proteobacteria, the progenitor lineage of the mitochondrion. The presence of the largest and central MICOS component Mic60 in α-proteobacteria indicates that MICOS has indeed a pre-endosymbiotic origin. Based on its overall structural conservation and the strong sequence conservation of the C-terminal mitofilin domain (Figure 3B; Table S2), the simplest hypothesis is that this protein works similarly in α-proteobacterial cytoplasmic membranes. Indeed, several α-proteobacterial groups are known for developing intracytoplasmic membranes (ICMs) of diverse morphologies. These include vesicular, tubular, plate-like, and thylakoid-like ICMs, some of which resemble the mitochondrial cristae of several eukaryotic lineages [32]. The discovery of the molecular bases of mitochondrial cristae morphogenesis through MICOS, together with our finding of its ancient α-proteobacterial origins, brings new life to the idea that mitochondrial cristae are homologous to α-proteobacterial ICMs (Figure 4).

MICOS is also required for proper MIM-MOM tethering and interacts with several outer membrane proteins, including the translocase of the outer mitochondrial membrane (TOM) complex, the sorting and assembly machinery (SAM) complex, and the voltage-dependent anion channel (VDAC) [2, 5, 8] (Figure 1). These interactions are required for both MIM-MOM tethering (contact sites) and the proper import and assembly of certain mitochondrial proteins [8, 12, 33]. While no obvious orthologs of the TOM complex or VDAC have been reported in bacteria, BamA, the major component of the beta-barrel assembly machinery (BAM) complex in Gram-negative bacteria, performs a similar function and is orthologous to Sam50, the major component of the SAM complex [34, 35]. Since the structures of α-proteobacterial Mic60 and Omp85 are similar to their homologs in mitochondria, we hypothesize that their interaction at mitochondrial contact sites is also an ancient feature that was inherited from the α-proteobacterial endosymbiont [2, 10, 33].

The presence of MICOS in most members of the α-proteobacteria, but not in the rickettsiales, is in agreement with suggestions that the mitochondrial ancestor was a metabolically versatile α-proteobacterium, similar to the purple non-sulfur bacteria [36]. Although it is conceivable that the rickettsiales lost MICOS during their transition to a parasitic life, our findings suggest that the α-proteobacterium that gave rise to the proto-mitochondrion already had the capability to remodel its cytoplasmic membrane as a result of an increasing bioenergetic demand.

Variations in Cristae Morphology Do Not Correlate with Presence of MICOS Subunits

Mitochondrial cristae morphology is relatively well conserved and has been utilized in taxonomy to delineate major eukaryotic groups [37]. However, the molecular bases for variations in cristae morphology are unknown. Given the importance of MICOS in cristae morphogenesis, we asked whether there is any correlation between the distribution of MICOS components and specific cristae morphotypes. We mapped both traits onto the eukaryote evolutionary tree (Figure 4). Although there is a perfect correlation between presence or absence of MICOS and that of cristae, there appears to be no correlation between the presence of specific MICOS components and the morphology of cristae in different eukaryote lineages. For example, archaeplastids have flat (lamellar) cristae, whereas alveolates and stramenopiles have tubular cristae, but in all three lineages, there are species that have the same core MICOS components (Mic60, Mic10, and Mic19). Similarly, animals and fungi have flat (lamellar) cristae [38] despite their MICOS complexes containing more subunits. Although euglenozoans appear to have lost Mic60, the heteroloboseans *Naegleria gruberi* and *Percolomonas* sp. retain Mic60 (Figure 2; Table S1), indicating that the defining discoidal cristae of discicristates are not correlated with a specific known MICOS subunit composition [38].

Conclusions

MICOS is an ancient eukaryotic multi-protein complex. Its conservation among eukaryotes attests to its critical role, whereas its evolutionary history exemplifies the coevolution of mitochondrial structure and function. The evidence presented here is indicative of the evolutionary continuity of a general mechanism to regulate membrane invaginations and organize membrane growth. We challenge the prevailing view that mitochondrial cristae have a post-endosymbiotic origin and provide evidence for the existence of an α-proteobacterial MICOS that may perform similar functions as mitochondrial MICOS. Future experimental studies will undoubtedly clarify the role of Mic60 in α-proteobacteria, extend MICOS protein composition in diverse eukaryotes, and deepen our understanding of the formation and evolution of cristae morphologies.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, three figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.04.006.

AUTHOR CONTRIBUTIONS

S.A.M.-G. and J.G.W. designed the study. S.A.M.-G., K.A.B., K.D.S., and J.G.W. performed experiments and analyzed data. S.A.M.-G., C.H.S., J.B.D., and J.G.W. wrote the paper.

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REFERENCES

1. von der Malsburg, K., Müller, J.M., Bohnert, M., Oeljeklaus, S., Kwiatkowska, P., Becker, T., Loniewska-Lwowska, A., Wiese, S., Rao, S., Milenkovic, D., et al. (2011). Dual role of mitofilin in mitochondrial membrane organization and protein biogenesis. Dev. Cell *21*, 694–707.
2. Harner, M., Körner, C., Walther, D., Mokranjac, D., Kaesmacher, J., Welsch, U., Griffith, J., Mann, M., Reggiori, F., and Neupert, W. (2011).

The mitochondrial contact site complex, a determinant of mitochondrial architecture. EMBO J. *30*, 4356–4370.

3. Hoppins, S., Collins, S.R., Cassidy-Stone, A., Hummel, E., Devay, R.M., Lackner, L.L., Westermann, B., Schuldiner, M., Weissman, J.S., and Nunnari, J. (2011). A mitochondrial-focused genetic interaction map reveals a scaffold-like complex required for inner membrane organization in mitochondria. J. Cell Biol. *195*, 323–340.
4. Pfanner, N., van der Laan, M., Amati, P., Capaldi, R.A., Caudy, A.A., Chacinska, A., Darshi, M., Deckers, M., Hoppins, S., Icho, T., et al. (2014). Uniform nomenclature for the mitochondrial contact site and cristae organizing system. J. Cell Biol. *204*, 1083–1086.
5. John, G.B., Shang, Y., Li, L., Renken, C., Mannella, C.A., Selker, J.M.L., Rangell, L., Bennett, M.J., and Zha, J. (2005). The mitochondrial inner membrane protein mitofilin controls cristae morphology. Mol. Biol. Cell *16*, 1543–1554.
6. Rabl, R., Soubannier, V., Scholz, R., Vogel, F., Mendl, N., Vasiljev-Neumeyer, A., Körner, C., Jagasia, R., Keil, T., Baumeister, W., et al. (2009). Formation of cristae and crista junctions in mitochondria depends on antagonism between Fcj1 and Su e/g. J. Cell Biol. *185*, 1047–1063.
7. Alkhaja, A.K., Jans, D.C., Nikolov, M., Vukotic, M., Lytovchenko, O., Ludewig, F., Schliebs, W., Riedel, D., Urlaub, H., Jakobs, S., and Deckers, M. (2012). MINOS1 is a conserved component of mitofilin complexes and required for mitochondrial function and cristae organization. Mol. Biol. Cell *23*, 247–257.
8. Bohnert, M., Wenz, L.-S., Zerbes, R.M., Horvath, S.E., Stroud, D.A., von der Malsburg, K., Müller, J.M., Oeljeklaus, S., Perschil, I., Warscheid, B., et al. (2012). Role of mitochondrial inner membrane organizing system in protein biogenesis of the mitochondrial outer membrane. Mol. Biol. Cell *23*, 3948–3956.
9. Vogel, F., Bornhövd, C., Neupert, W., and Reichert, A.S. (2006). Dynamic subcompartmentalization of the mitochondrial inner membrane. J. Cell Biol. *175*, 237–247.
10. Mannella, C.A. (2006). Structure and dynamics of the mitochondrial inner membrane cristae. Biochim. Biophys. Acta *1763*, 542–548.
11. Herrmann, J.M. (2011). MINOS is plus: a Mitofilin complex for mitochondrial membrane contacts. Dev. Cell *21*, 599–600.
12. Zerbes, R.M., Bohnert, M., Stroud, D.A., von der Malsburg, K., Kram, A., Oeljeklaus, S., Warscheid, B., Becker, T., Wiedemann, N., Veenhuis, M., et al. (2012). Role of MINOS in mitochondrial membrane architecture: cristae morphology and outer membrane interactions differentially depend on mitofilin domains. J. Mol. Biol. *422*, 183–191.
13. Head, B.P., Zulaika, M., Ryazantsev, S., and van der Bliek, A.M. (2011). A novel mitochondrial outer membrane protein, MOMA-1, that affects cristae morphology in Caenorhabditis elegans. Mol. Biol. Cell *22*, 831–841.
14. Darshi, M., Mendiola, V.L., Mackey, M.R., Murphy, A.N., Koller, A., Perkins, G.A., Ellisman, M.H., and Taylor, S.S. (2011). ChChd3, an inner mitochondrial membrane protein, is essential for maintaining crista integrity and mitochondrial function. J. Biol. Chem. *286*, 2918–2932.
15. An, J., Shi, J., He, Q., Lui, K., Liu, Y., Huang, Y., and Sheikh, M.S. (2012). CHCM1/CHCHD6, novel mitochondrial protein linked to regulation of mitofilin and mitochondrial cristae morphology. J. Biol. Chem. *287*, 7411–7426.
16. Weber, T.A., Koob, S., Heide, H., Wittig, I., Head, B., van der Bliek, A., Brandt, U., Mittelbronn, M., and Reichert, A.S. (2013). APOOL is a cardiolipin-binding constituent of the Mitofilin/MINOS protein complex determining cristae morphology in mammalian mitochondria. PLoS ONE *8*, e63683.
17. Adl, S.M., Simpson, A.G.B., Lane, C.E., Lukeš, J., Bass, D., Bowser, S.S., Brown, M.W., Burki, F., Dunthorn, M., Hampl, V., et al. (2012). The revised classification of eukaryotes. J. Eukaryot. Microbiol. *59*, 429–493.
18. Odgren, P.R., Toukatly, G., Bangs, P.L., Gilmore, R., and Fey, E.G. (1996). Molecular characterization of mitofilin (HMP), a mitochondria-associated protein with predicted coiled coil and intermembrane space targeting domains. J. Cell Sci. *109*, 2253–2264.
19. Mannella, C.A., Marko, M., Penczek, P., Barnard, D., and Frank, J. (1994). The internal compartmentation of rat-liver mitochondria: tomographic study using the high-voltage transmission electron microscope. Microsc. Res. Tech. *27*, 278–283.
20. Nicastro, D., Frangakis, A.S., Typke, D., and Baumeister, W. (2000). Cryo-electron tomography of neurospora mitochondria. J. Struct. Biol. *129*, 48–56.
21. Deng, Y., Marko, M., Buttle, K.F., Leith, A., Mieczkowski, M., and Mannella, C.A. (1999). Cubic membrane structure in amoeba (Chaos carolinensis) mitochondria determined by electron microscopic tomography. J. Struct. Biol. *127*, 231–239.
22. Embley, T.M. (2006). Multiple secondary origins of the anaerobic lifestyle in eukaryotes. Philos. Trans. R. Soc. Lond. B Biol. Sci. *361*, 1055–1067.
23. Zierdt, C.H., Donnolley, C.T., Muller, J., and Constantopoulos, G. (1988). Biochemical and ultrastructural study of Blastocystis hominis. J. Clin. Microbiol. *26*, 965–970.
24. Uni, S., Iseki, M., Maekawa, T., Moriya, K., and Takada, S. (1987). Ultrastructure of Cryptosporidium muris (strain RN 66) parasitizing the murine stomach. Parasitol. Res. *74*, 123–132.
25. Mogi, T., and Kita, K. (2010). Diversity in mitochondrial metabolic pathways in parasitic protists Plasmodium and Cryptosporidium. Parasitol. Int. *59*, 305–312.
26. Keithly, J.S., Langreth, S.G., Buttle, K.F., and Mannella, C.A. (2005). Electron tomographic and ultrastructural analysis of the Cryptosporidium parvum relict mitochondrion, its associated membranes, and organelles. J. Eukaryot. Microbiol. *52*, 132–140.
27. Olichon, A., Baricault, L., Gas, N., Guillou, E., Valette, A., Belenguer, P., and Lenaers, G. (2003). Loss of OPA1 perturbates the mitochondrial inner membrane structure and integrity, leading to cytochrome c release and apoptosis. J. Biol. Chem. *278*, 7743–7746.
28. Oka, T., Sayano, T., Tamai, S., Yokota, S., Kato, H., Fujii, G., and Mihara, K. (2008). Identification of a novel protein MICS1 that is involved in maintenance of mitochondrial morphology and apoptotic release of cytochrome c. Mol. Biol. Cell *19*, 2597–2608.
29. Purkanti, R., and Thattai, M. (2015). Ancient dynamin segments capture early stages of host-mitochondrial integration. Proc. Natl. Acad. Sci. USA *112*, 2800–2805.
30. Lynch, M. (2012). The evolution of multimeric protein assemblages. Mol. Biol. Evol. *29*, 1353–1366.
31. Williams, K.P., Sobral, B.W., and Dickerman, A.W. (2007). A robust species tree for the alphaproteobacteria. J. Bacteriol. *189*, 4578–4586.
32. Drews, G., and Golecki, J.R. (1995). Structure, molecular organization, and biosynthesis of membranes of purple bacteria. In Anoxygenic Photosynthetic Bacteria: Advances in Photosynthesis and Respiration, R.E. Blankenship, M.T. Madigan, and C.E. Bauer, eds. (Springer), pp. 231–257.
33. Körner, C., Barrera, M., Dukanovic, J., Eydt, K., Harner, M., Rabl, R., Vogel, F., Rapaport, D., Neupert, W., and Reichert, A.S. (2012). The C-terminal domain of Fcj1 is required for formation of crista junctions and interacts with the TOB/SAM complex in mitochondria. Mol. Biol. Cell *23*, 2143–2155.
34. Paschen, S.A., Waizenegger, T., Stan, T., Preuss, M., Cyrklaff, M., Hell, K., Rapaport, D., and Neupert, W. (2003). Evolutionary conservation of biogenesis of β-barrel membrane proteins. Nature *426*, 862–866.
35. Gentle, I., Gabriel, K., Beech, P., Waller, R., and Lithgow, T. (2004). The Omp85 family of proteins is essential for outer membrane biogenesis in mitochondria and bacteria. J. Cell Biol. *164*, 19–24.
36. Atteia, A., Adrait, A., Brugière, S., Tardif, M., van Lis, R., Deusch, O., Dagan, T., Kuhn, L., Gontero, B., Martin, W., et al. (2009). A proteomic survey of Chlamydomonas reinhardtii mitochondria sheds new light on the metabolic plasticity of the organelle and on the nature of the alpha-proteobacterial mitochondrial ancestor. Mol. Biol. Evol. *26*, 1533–1548.
37. Taylor, F.J.R. (1976). Flagellate phylogeny: a study in conflicts. J. Protozool. *23*, 28–40.
38. Patterson, D.J. (1999). The diversity of eukaryotes. Am. Nat. *154* (S4), S96–S124.