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## Mitofilin complexes

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## Review

Ralf M. Zerbes, Ida J. van der Klei, Marten Veenhuis, Nikolaus Pfanner, Martin van der Laan\* and Maria Bohnert

# Mitofilin complexes: conserved organizers of mitochondrial membrane architecture

**Abstract:** Mitofilin proteins are crucial organizers of mitochondrial architecture. They are located in the inner mitochondrial membrane and interact with several protein complexes of the outer membrane, thereby generating contact sites between the two membrane systems of mitochondria. Within the inner membrane, mitofilins are part of hetero-oligomeric protein complexes that have been termed the mitochondrial inner membrane organizing system (MINOS). MINOS integrity is required for the maintenance of the characteristic morphology of the inner mitochondrial membrane, with an inner boundary region closely apposed to the outer membrane and cristae membranes, which form large tubular invaginations that protrude into the mitochondrial matrix and harbor the enzyme complexes of the oxidative phosphorylation machinery. MINOS deficiency comes along with a loss of crista junction structures and the detachment of cristae from the inner boundary membrane. MINOS has been conserved in evolution from unicellular eukaryotes to humans, where alterations of MINOS subunits are associated with multiple pathological conditions.

**Keywords:** cristae; crista junction; Fcjl1; MINOS; Mio10; mitochondrial morphology.

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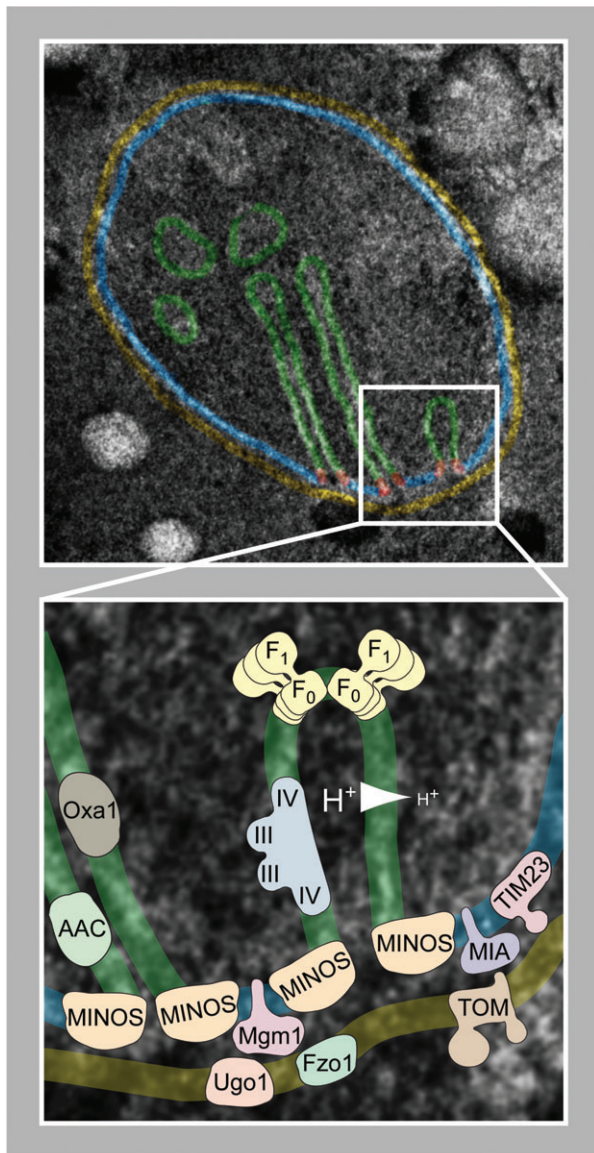
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## Introduction: mitofilins as key players in mitochondrial membrane organization

Mitochondria are complex organelles of eukaryotic cells that form dynamic tubular structures organized into extended, highly branched networks. The overall architecture of mitochondria is characterized by the presence of two separate membrane systems with different functions. The outer membrane forms the border between the mitochondrial interior and the cytosol and mediates the communication of mitochondria with the cellular environment. The inner membrane is a heterogeneous structure composed of morphologically distinct subdomains, the inner boundary membrane and the cristae (Figure 1). The inner boundary membrane is in close proximity to the outer membrane, and both membranes together confine the mitochondrial intermembrane space. Cristae membranes are large invaginations that extend from the inner boundary membrane into the mitochondrial matrix. Cristae are variable in size and shape and can appear as flat lamellae, extended tubules or heterogeneous membrane sacs (Perkins et al., 1997; Mannella et al., 2001; Frey et al., 2002; Zick et al., 2009). The connections between the two subdomains of the inner membrane have been termed crista junctions (Figure 1) (Perkins et al., 1997; Frey and Mannella, 2000; Renken et al., 2002). In contrast to the polymorphic cristae, crista junctions have a relatively uniform appearance. Typically, they form short tubules with a diameter of a few ten nanometers (Frey and Mannella, 2000). How these characteristic structures are formed and maintained despite the forces exerted by the



**Figure 1** Mitochondrial membrane organization.

A cryo-electron microscopy image of a *Saccharomyces cerevisiae* wild-type mitochondrion is shown to illustrate key features of mitochondrial membrane architecture. Mitochondria are surrounded by two membranes, the outer membrane (yellow) and the inner membrane, which consists of different domains: the inner boundary membrane (blue), which is closely apposed to the outer membrane, and the cristae (green). Inner boundary and cristae membranes are connected by narrow tubular openings, the crista junctions (red). Proteins of the inner membrane are unevenly distributed between the different domains. The proton-pumping respiratory chain complexes III and IV, ADP/ATP carrier (AAC), Oxa1, and  $F_1F_0$ -ATP synthase are mainly located in cristae membranes. Proteins and complexes involved in functions connected to both mitochondrial membranes, such as TIM23, Mgm1 and Mia40 (MIA), are preferentially found in the inner boundary membrane in proximity to their outer membrane partner proteins and complexes (TOM, Fzo1 and Ugo1). MINOS is required for the formation and/or maintenance of crista junction structures and inner membrane-outer membrane contact sites.

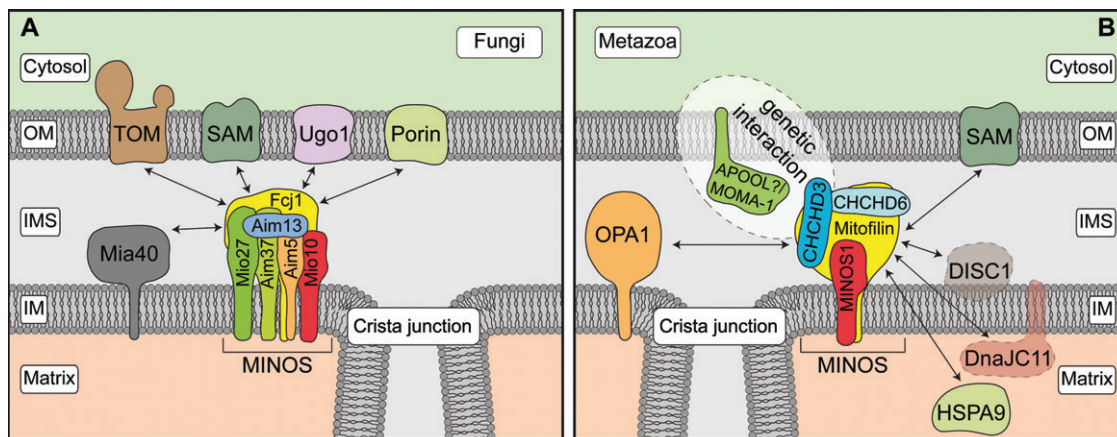
high membrane curvature has been a major unresolved question in the field of mitochondrial biology.

An important step towards understanding the molecular basis of inner membrane architecture was the identification of mitofilin (IMMT, MINOS2), a protein required for the maintenance of crista junctions. Originally termed HMP (heart muscle protein) because of its abundance in the human heart (Icho et al., 1994), mitofilin was subsequently recognized as a protein of the mitochondrial inner membrane (Figure 2) (Odgren et al., 1996; Gieffers et al., 1997). Mitofilin possesses an N-terminal mitochondrial targeting sequence followed by a transmembrane segment and a large domain exposed to the intermembrane space. The intermembrane space domain consists of at least two defined subdomains: an extended putative coiled-coil region and a short C-terminal mitofilin signature domain (Rabl et al., 2009; von der Malsburg et al., 2011; Körner et al., 2012; Zerbes et al., 2012). Mitofilins are ubiquitous mitochondrial proteins that share this domain organization from yeast to humans, whereas the mitofilin signature domain is the only part of the protein that exhibits strong conservation at the primary structure level across species (Rabl et al., 2009; Körner et al., 2012; Zerbes et al., 2012). The *Saccharomyces cerevisiae* mitofilin protein (also termed formation of crista junctions 1/Fcj1) was the first protein shown to be mainly located at crista junctions (Rabl et al., 2009; Harner et al., 2011). Depletion of mitofilin in human cells and worms or deletion of mitofilin in yeast leads to an extension of the inner membrane surface, a massive loss of crista junctions and to abnormal crista structures that appear as stacked lamellae disconnected from the inner boundary membrane (John et al., 2005; Rabl et al., 2009; Mun et al., 2010; Harner et al., 2011; Hoppins et al., 2011; von der Malsburg et al., 2011; Alkhaja et al., 2012). These observations point to a conserved function of mitofilin in the formation and/or maintenance of crista junctions.

## Structural and functional links of crista junctions and membrane contact sites

Although crista junctions are ubiquitous structural elements of mitochondria, their role for mitochondrial function is not fully understood. Their defined shape and narrow diameter suggest that they might act as barriers between the intracristae lumen and the boundary





**Figure 2** MINOS is a conserved organizer of mitochondrial membranes.

The MINOS complex is located in the inner mitochondrial membrane (IM), preferentially at crista junctions, and exposes domains to the intermembrane space (IMS). (A) In fungi, MINOS is composed of at least six different proteins (mitofilin/Fc11, Mio10, Mio27, Aim5, Aim13 and Aim37) and is connected to the outer membrane (OM) complexes TOM, SAM, Porin and Ugo1. Mitofilin also acts as an adaptor between the intermembrane space import protein Mia40 and the TOM complex, thereby supporting the import of intermembrane space proteins. (B) In metazoa, MINOS has been reported to include MINOS1, mitofilin (MINOS2, IMMT), CHCHD3 (MINOS3, CHCH-3) and CHCHD6 (CHCM1). In *Caenorhabditis elegans*, genetic interactions between mitofilin/IMMT-1, CHCH-3 and MOMA-1 (a protein related to human APOOL) have been reported. Suggested interaction partners of metazoan MINOS are the outer membrane SAM complex, the inner membrane morphology protein OPA1, the chaperones HSPA9 and DnaJC11 and disrupted in schizophrenia 1 (DISC1), a protein connected to the development of mental disorders. The shown submitochondrial localization of DnaJC11 and DISC1 is hypothetical.

intermembrane space (Frey and Mannella, 2000; Mannella, 2006). Theoretical calculations indeed predict an imbalance of solute concentrations between these two subcompartments (Mannella et al., 2001). Furthermore, several studies reported on an uneven distribution of integral membrane proteins between inner boundary membrane and cristae membranes, suggesting that these morphologically distinct inner membrane regions are functionally specialized (Gilkerson et al., 2003; Vogel et al., 2006; Wurm and Jakobs, 2006; Suppanz et al., 2009; Stoldt et al., 2012). Cristae membranes are enriched in respiratory chain complexes,  $F_1F_0$ -ATP synthase, ADP/ATP carrier and Oxa1, a protein required for biogenesis of mitochondrially encoded subunits of respiratory chain complexes (Figure 1) (Gilkerson et al., 2003; Vogel et al., 2006; Wurm and Jakobs, 2006; Stoldt et al., 2012). These membrane regions are the main sites of ATP production in mitochondria (Gilkerson et al., 2003; Strauss et al., 2008; Davies et al., 2011). In contrast, the inner boundary membrane mainly contains protein complexes that are involved in the functional cooperation between inner and outer mitochondrial membranes, like the machineries for the import of nuclear-encoded mitochondrial precursor proteins (Figure 1) (Vogel et al., 2006; Wurm and Jakobs, 2006). The translocase of the inner membrane 23 (TIM23 complex, presequence translocase) mediates the import of precursor proteins that contain N-terminal

mitochondrial targeting signals (presequences) into the inner membrane and the matrix (Dolezal et al., 2006; Neupert and Herrmann, 2007; Chacinska et al., 2009; Endo et al., 2011). During the transport of presequence-containing precursor proteins, TIM23 physically interacts with the translocase of the outer membrane (TOM complex), thereby allowing for a direct handover of incoming precursors from the outer to the inner mitochondrial membrane (Schleyer and Neupert, 1985; Dekker et al., 1997; Chacinska et al., 2003, 2005; Mokranjac et al., 2005; Tamura et al., 2009).

Transient membrane interactions have also been observed in other processes that require the orchestrated action of outer and inner membrane protein complexes. When two mitochondria fuse with each other, the dynamin-like GTPases Fzo1 (outer membrane) and Mgm1 (inner membrane) act in concert (Okamoto and Shaw, 2005; Hoppins et al., 2007; Westermann, 2010; Palmer et al., 2011). This ensures the coordinated fusion of outer and inner membranes and prevents the accumulation of abnormal fusion intermediates. The outer membrane protein Ugo1 interacts with both Mgm1 and Fzo1, thus linking the fusion machineries of inner and outer mitochondrial membranes (Figure 1) (Sesaki and Jensen, 2004; Hoppins et al., 2009). In addition to such transient functional interactions, the inner boundary membrane appears to be anchored to the outer

membrane by persistent, structurally defined contact sites (Reichert and Neupert, 2002). The existence of such contact sites was first proposed by Hackenbrock 1968. In this study, isolated mitochondria were diluted into a buffer of high osmolarity, which leads to the extrusion of water and shrinking of the mitochondrial matrix compartment. Electron microscopy images showed that, under these conditions, the inner boundary membrane was largely dissociated from the outer membrane, but specific membrane regions remained tightly tethered. These morphologically defined contact sites have been proposed to depend on two-membrane-bridging scaffold-like complexes, but the identification of such complexes proved to be challenging (Reichert and Neupert, 2002).

Interestingly, a number of recent studies have reported on extensive interactions between several outer membrane protein complexes and the inner membrane mitofilin protein. In human cells, mitofilin was found to interact with the sorting and assembly machinery of the outer membrane (SAM complex) that has a central role in the biogenesis of outer membrane proteins (Figure 2B) (Xie et al., 2007; Darshi et al., 2011; Alkhaja et al., 2012; Ott et al., 2012). In yeast, interactions of mitofilin-containing protein complexes of the inner membrane with the SAM complex, the TOM complex, porin channels and Ugo1 have been reported (Figure 2A) (Harner et al., 2011; Hoppins et al., 2011; von der Malsburg et al., 2011; Körner et al., 2012; Zerbes et al., 2012). The interaction of mitofilin with the TOM complex was shown to support the import of precursor proteins into the intermembrane space via the mitochondrial intermembrane space assembly (MIA) machinery (von der Malsburg et al., 2011). Thus, mitofilin appears to be involved in the formation of both crista junctions and contact sites, two basic elements of mitochondrial membrane architecture. These findings have prompted the hypothesis of a structural and functional connection between contact sites and crista junctions. The possibility of such an overlapping localization has been repeatedly addressed, although never comprehensively clarified (van der Klei et al., 1994; Perkins et al., 1997; Reichert and Neupert, 2002; Harner et al., 2011; Körner et al., 2012). Some studies have suggested that the integrity of crista junctions may depend on their anchoring to the outer mitochondrial membrane via mitofilin-containing contact sites (Harner et al., 2011; Körner et al., 2012; Ott et al., 2012). Along the same lines, downregulation of the SAM complex, one of the outer membrane interaction partners of mitofilin, was found to result in alterations of mitochondrial ultrastructure in human cultured cells and yeast (Körner et al., 2012; Ott et al., 2012). A

detailed structure-function analysis of the yeast mitofilin protein led to the isolation of a truncated variant that interacts efficiently with both the SAM and TOM complexes in the outer membrane, whereas crista junctions are virtually lost (Zerbes et al., 2012). This mutant form of mitofilin lacks the C-terminal 49 amino acids of the protein that correspond to the highly conserved mitofilin domain. These data indicated that anchoring of mitofilin to the outer membrane is not sufficient for crista junction formation and that the role of mitofilin for crista junction formation likely involves further proteins. Indeed, earlier work had already suggested that mitofilin proteins are part of very large protein complexes, but the molecular composition of these structures had remained enigmatic (John et al., 2005; Rabl et al., 2009; Mun et al., 2010).

## Mitochondrial inner membrane organizing system (MINOS)

Several recent studies have reported on the discovery of a multi-subunit protein complex of the mitochondrial inner membrane in *S. cerevisiae* that contains mitofilin as a central component (Harner et al., 2011; Hoppins et al., 2011; von der Malsburg et al., 2011; Alkhaja et al., 2012). This protein complex was named the mitochondrial inner membrane organizing system (MINOS) or, alternatively, mitochondrial organizing structure (MitOS) or mitochondrial contact site (MICOS) complex (for an overview of the nomenclature see Herrmann, 2011). Intriguingly, quite different experimental approaches – biochemical isolation of mitofilin complexes by affinity chromatography, enrichment of outer/inner membrane contact sites and a systematic analysis of genetic interactions – led to the identification of the same protein complex composed of at least six different subunits: mitofilin/Fcj1, Mio10/MINOS1 (Mos1, Mcs10), Aim5 (Mcs12), Aim13 (Mcs19), Aim37 (Mcs27) and Mio27 (Mos2, Mcs29) (Table 1, Figure 2A). All subunits of the MINOS complex, which according to gel filtration experiments has a molecular mass of several megaDalton, are either integral or peripheral inner membrane proteins and expose their bulk domains to the intermembrane space (Harner et al., 2011; Hoppins et al., 2011; von der Malsburg et al., 2011). Remarkably, the core components of MINOS are highly conserved in evolution, which suggests considerable similarities in the mechanistic role of mitofilin-containing protein complexes for crista junction and membrane contact site formation in different organisms (Figure 2).

MINOS yeast	ORF name	Mitochondrial ultrastructure in $\Delta$ mutants	Protein characteristics	MINOS metazoa
Fcj1	YKR016W	Virtually no crista junctions; increased inner membrane surface	Integral membrane protein of 58.3 kDa; C-terminal signature domain	Mitofilin/ MINOS2/ IMMT
Mio10 (Mcs10, Mos1)	YCL057C-A	Virtually no crista junctions; increased inner membrane surface	Integral membrane protein of 10.4 kDa; multiple GxxxG helix packing motifs	MINOS1
Mio27 (Mcs29, Mos2)	YGR235C	Minor effects on cristae morphology	Integral membrane proteins of 26.9 kDa (Mio27) and 27.0 kDa (Aim37); share 17.4% identity and 41.3% similarity	APOOL/ MOMA-1
Aim37 (Mcs27)	YNL100W	Decreased number of crista junctions		
Aim13 (Mcs19)	YFR011C	Decreased number of crista junctions; bifurcated crista junctions	Peripheral inner membrane protein of 18.9 kDa; modified CHCH domain	CHCHD3/ MINOS3/ CHCH-3; CHCHD6/ CHCM1
Aim5 (Mcs12)	YBR262C	Decreased number of crista junctions	Integral membrane protein of 12.4 kDa	

**Table 1** Subunit composition of MINOS.

Six different subunits of MINOS have been identified in fungi, most of which are evolutionary conserved.  $\Delta$  mutants, strain with a deletion of the respective yeast gene.

## Mio10/MINOS1

Mio10 is a small transmembrane protein crucial for MINOS stability. Deletion of *MIO10* in yeast leads to reduced cell growth, the absence of assembled MINOS complexes and dramatic alterations of inner membrane architecture, including the formation of stacked, lamellar cristae and loss of crista junctions (Harner et al., 2011; Hoppins et al., 2011; von der Malsburg et al., 2011; Alkhaja et al., 2012). The morphological alterations observed in mitochondria of *mio10 $\Delta$*  cells are similar to those seen in mitofilin mutants, indicating that mitofilin and Mio10 are the core components of MINOS. The human Mio10 ortholog MINOS1 (28.4% identity, 45.1% similarity to *S. cerevisiae* Mio10; von der Malsburg et al., 2011) has recently been described as a mitochondrial inner membrane protein (Figure 2B) (Alkhaja et al., 2012). Like Mio10, MINOS1 has two predicted transmembrane segments connected by a short, positively charged loop. As assessed by co-immunoprecipitation and gel filtration, MINOS1 is part of a protein complex in the megaDalton range and interacts with mitofilin (Alkhaja et al., 2012). Mio10/MINOS1 contains highly conserved glycine-rich motifs in both predicted transmembrane helices that are likely important

for helix packing (Russ and Engelman, 2000; Alkhaja et al., 2012). These features suggest that Mio10/MINOS1 may form oligomers that could be part of the structural backbone of MINOS complexes at crista junctions.

## Aim5

Aim5 is an integral inner membrane protein with one predicted transmembrane segment and a soluble domain exposed to the intermembrane space (Harner et al., 2011; Hoppins et al., 2011; von der Malsburg et al., 2011). Aim5 is the only subunit of the yeast MINOS complex, for which no orthologs have been found so far outside the fungal kingdom. Deletion of the *AIM5* gene leads to a partial destabilization of MINOS and a reduced number of crista junctions. Regions with stacked, lamellar cristae are found in Aim5-deficient mitochondria, but less frequently compared to *mio10 $\Delta$*  or *fcj1 $\Delta$*  mutant mitochondria. *AIM5* as well as two other genes encoding mitofilin/Fcj1-interacting proteins (*AIM13* and *AIM37*) have originally been identified in *S. cerevisiae* as genes that are involved in the correct distribution of mitochondria from mother to daughter cells during the budding

process (Hess et al., 2009). In this genetic screen for altered inheritance of mitochondria (AIM), a direct or indirect role in mitochondrial biogenesis and distribution has been attributed to over 100 different gene products, more than half of which previously had no assigned function. The Aim proteins include factors involved in mitochondrial migration, several metabolic pathways, respiratory chain assembly and mitochondrial morphology. Notably, the *FCJ1* gene was also identified in the same screen and therefore received the alternative name *AIM28* (Hess et al., 2009).

## Aim13/CHCH-3/CHCHD3 and CHCHD6

Yeast mitochondria lacking Aim13 possess a partially collapsed MINOS complex and exhibit alterations in inner membrane architecture similar to *aim5Δ* mitochondria (Harner et al., 2011; Hoppins et al., 2011; von der Malsburg et al., 2011). Additionally, *aim13Δ* mitochondria often show abnormal crista junctions that appear bifurcated in cross sections, a phenomenon that is not commonly observed in wild-type mitochondria (Harner et al., 2011). Aim13 is the only MINOS subunit that lacks obvious membrane-spanning segments. Instead, it has a predicted N-myristoylation site and is peripherally associated to the intermembrane space side of the inner membrane (Harner et al., 2011; Hoppins et al., 2011; von der Malsburg et al., 2011). Human mitofilin was shown to interact with two different putatively myristoylated peripheral inner membrane proteins located in the intermembrane space, the twin CX<sub>2</sub>C proteins CHCHD3 (MINOS3) and CHCHD6 (CHCM1) (Figure 2B) (Xie et al., 2007; Darshi et al., 2011; Alkhaja et al., 2012; An et al., 2012; Ott et al., 2012). Twin CX<sub>2</sub>C proteins are characterized by a cysteine-rich motif that forms two disulfide bonds required for stabilization of their eponymous coiled coil-helix-coiled coil-helix (CHCH) domain (Cavallaro, 2010). CHCHD6 and CHCHD3 have similar sizes (235 and 227 amino acids, respectively), corresponding domain organizations with the CHCH fold near the C-terminus and an overall sequence identity of 36% (Cavallaro, 2010; An et al., 2012). Knockdown of either protein in cultured cells results in similar phenotypes, including reduced growth rates, impaired mitochondrial function and loss of mitofilin.

On the level of mitochondrial ultrastructure, depletion of CHCHD3 or CHCHD6 leads to drastic changes in cristae architecture, including fragmentation, rounding

and overall loss of cristae membranes, as well as narrowing of crista junctions in the case of CHCHD3 (Darshi et al., 2011; An et al., 2012). CHCHD3 was found in a complex with mitofilin and MINOS1 (Darshi et al., 2011; Alkhaja et al., 2012) but also was suggested to interact with the OPA1 (optic atrophy 1) protein (Darshi et al., 2011). Human OPA1 is a dynamin-like GTPase involved in mitochondrial fusion that is homologous to the yeast Mgm1 protein (see above). Both OPA1 and Mgm1 have also been implicated in cristae formation (Frezza et al., 2006; Meeusen et al., 2006; Yamaguchi et al., 2008). The *Caenorhabditis elegans* CHCHD3 homolog CHCH-3 has been identified in an RNAi screen for altered mitochondrial morphology (Head et al., 2011). CHCH-3 contains the so-called domain of unknown function (DUF) 1690, which is also found in fungal proteins. The *S. cerevisiae* DUF1690-containing protein is Aim13 (Cavallaro, 2010), and homology searches using BLAST identify Aim13 as the closest CHCH-3 homolog in *S. cerevisiae*. Aim13 does not possess a classical CHCH domain, but a single CX<sub>10</sub>C motif, which aligns with the cysteines 2 and 3 of the CHCH domains in CHCHD3/CHCHD6 proteins. This indicates that Aim13 may contain a modified CHCH-fold with only one disulfide bond (Cavallaro, 2010) and suggests a cognate function of Aim13 and CHCHD3/CHCHD6 proteins.

## Mio27/Aim37/MOMA-1/APOOL

The aforementioned RNAi screen that identified CHCH-3 as a component required for mitochondrial ultrastructure in *C. elegans* yielded two more candidates with very similar morphology phenotypes: the *C. elegans* mitofilin ortholog IMMT-1 and MOMA-1 (Figure 2B) (Head et al., 2011). When one of these components is depleted, severe changes in the overall mitochondrial shape are observed. Both *moma-1* and *immt-1* mutants additionally exhibit altered mitochondrial ultrastructures with dilated cristae and occasional concentric inner membrane whorls. MOMA-1 has similarity to the human mitochondrial protein APOOL (Apo-O-like/CXorf33) and the related Apolipoprotein O/My025 (Lamant et al., 2006; Head et al., 2011; Ott et al., 2012). Yeast cells express two proteins related to MOMA-1, Aim37 and Mio27, both of which are subunits of the MINOS complex (Figure 2A) (Head et al., 2011; van der Laan et al., 2012). Aim37 and Mio27 are integral inner mitochondrial membrane proteins with two predicted transmembrane segments each. Despite their homology, the two proteins appear to be functionally



distinct. Deletion of *AIM37* leads to destabilization of MINOS and loss of crista junctions, whereas deletion of *MIO27* has no apparent effects on MINOS integrity and mitochondrial ultrastructure (Harner et al., 2011; Hoppins et al., 2011; von der Malsburg et al., 2011). Interestingly, *C. elegans* MOMA-1 has been localized mainly to the outer mitochondrial membrane (although it was suggested that a fraction of MOMA-1 may be located in the inner membrane) (Head et al., 2011). It is so far unknown whether IMMT-1 and MOMA-1 physically interact, but their genetic interaction suggests that they act in a concerted manner to control mitochondrial membrane architecture (Figure 2B).

## MINOS as a central organizer of mitochondrial architecture and function

Although the mechanistic functions of the different MINOS components for mitochondrial membrane organization remain to be elucidated, several features of MINOS have been described in yeast that indicate a role of this protein complex in inner membrane scaffolding. (i) Submitochondrial localization by immunogold labeling shows that the MINOS subunits are predominantly located in the inner boundary membrane, especially in close proximity to crista junctions (Figure 1) (Rabl et al., 2009; Harner et al., 2011). (ii) Microscopic studies using GFP-tagged MINOS subunits suggest that MINOS consists of defined punctae in the mitochondrial periphery connected by extended filamentous structures that circumscribe the mitochondrial tubules (Hoppins et al., 2011). This specific arrangement suggests a skeleton-like function of MINOS for the maintenance of the mitochondrial inner membrane shape. (iii) Analysis of MINOS integrity in mutants lacking different components of the complex revealed a striking correlation between overall MINOS stability and maintenance of crista junctions (von der Malsburg et al., 2011). However, MINOS not only functions as a membrane scaffold but is also involved in mitochondrial protein biogenesis. The role of the MINOS core subunit mitofilin/Fcj1 in the import of small cysteine-rich proteins into the intermembrane space appears to be independent of its function in inner membrane morphogenesis. In this process, mitofilin is thought to act as an adaptor that links the inner membrane import receptor Mia40 to the outer membrane TOM complex (von der Malsburg et al., 2011). Moreover, MINOS interacts with the SAM complex, which together with the TOM complex

mediates the biogenesis of outer membrane  $\beta$ -barrel proteins. Although a role of MINOS in  $\beta$ -barrel protein import and assembly has not been established so far (Körner et al., 2012), it seems possible that MINOS is involved in the spatial organization and functional cooperation of the TOM and SAM complexes.

Several lines of evidence suggest that MINOS and its interaction partners constitute a branch of a much larger membrane-organizing system (van der Laan et al., 2012). The endoplasmic reticulum (ER)-mitochondria encounter structure (ERMES) tethers the outer mitochondrial membrane to the endoplasmic reticulum membrane and has been suggested to facilitate the transport of phospholipids between both organelles (Kornmann et al., 2009, 2011; Stroud et al., 2011). MINOS and ERMES are linked through multiple genetic interactions, indicating that these membrane-bridging complexes are functionally connected (Hoppins et al., 2011). Moreover, the ERMES complex shares at least one subunit (Mdm10) with the SAM complex, which physically interacts with MINOS (Meisinger et al., 2004, 2006). These findings suggest that MINOS, ERMES, SAM and likely further machineries involved in membrane morphology, biogenesis and communication form a large, dynamic ER-mitochondria organizing network (ERMIONE) that spans three membranes and integrates different metabolic and developmental signals to coordinate the architecture, biogenesis and function of mitochondria.

## Implications of MINOS functions for human disease

Mitochondrial architecture is important for crucial mitochondrial functions, including ATP generation via oxidative phosphorylation (OXPHOS), calcium homeostasis and programmed cell death (apoptosis) (Frey and Mannella, 2000; Frezza et al., 2006; Clapham, 2007; Strauss et al., 2008; Rizzuto et al., 2009; Martins de Brito and Scorrano, 2010; Davies et al., 2011; Green et al., 2011). Accordingly, alterations of mitochondrial morphology are a hallmark of numerous human diseases, ranging from myopathies, neuropathies and neurodegenerative disorders to metabolic diseases and different types of cancer. Well-known examples for proteins involved in mitochondrial dynamics and human disease are the components of the mitochondrial fusion and fission machineries, which have been the topic of extensive research over the last years (Okamoto and Shaw, 2005; Hoppins et al., 2007; Westermann, 2010; Palmer et al., 2011). Alterations of the mitochondrial



fusion and fission machineries are linked to several forms of inherited diseases. The membrane fusion GTPases OPA1 and mitofusin 2 (MFN2) are frequently found mutated in cases of dominant optic atrophy (DOA) and Charcot-Marie-Tooth disease type 2A, respectively, two distinct types of neuropathies (Alexander et al., 2000; Delettre et al., 2000; Züchner et al., 2004; Chen and Chan, 2006; Olichon et al., 2006; Chen et al., 2007; Feely et al., 2011). Mutations affecting the mitochondrial fission factor dynamin-related protein 1 (DRP1) are connected to cardiomyopathy and to a neonatal lethal case of multisymptomatic disease with microencephaly and optic atrophy (Detmer and Chan, 2007; Waterham et al., 2007; Ashrafian et al., 2010; Westermann, 2010). The connection between fusion/fission proteins and the pathogenesis of the diseases is not fully understood. One central aspect may be that defects in the mitochondrial fusion machinery impair the mixing of contents between different mitochondrial particles, a process that is critical for the maintenance of a healthy, functional mitochondrial population (Nakada et al., 2001, 2009; Chen et al., 2007, 2010; Detmer and Chan, 2007). Mitochondrial fission is thought to be a prerequisite for the distribution of mitochondria to neuronal axon terminals, explaining why defects in mitochondrial fission and intracellular transport of mitochondria are associated with neurodegenerative phenotypes (Li et al., 2004; Verstreken et al., 2005; Baloh et al., 2007; Detmer and Chan, 2007; Chen and Chan, 2009; Westermann, 2010; Saxton and Hollenbeck, 2012).

Apart from the overall shape and dynamics of the mitochondrial network governed by the fusion and fission machineries, disorganization of mitochondrial ultrastructure has emerged as a hallmark of many disease states. The impact of altered mitochondrial inner membrane architecture on pathogenetic processes is far from understood at the molecular level; however, conspicuous changes in the frequency and shape of cristae membrane domains have been observed in numerous pathologies (Trimmer et al., 2000; Acehan et al., 2007; Zick et al., 2009; Arismendi-Morillo, 2011). Considering the role of MINOS for the formation of crista junctions and maintenance of normal cristae morphology, it is tempting to speculate that defects in central MINOS components will lead to profound disturbances of mitochondrial functions and, ultimately, to pathophysiological situations. The characteristic shape of the inner mitochondrial membrane is believed to optimize the performance of the OXPHOS machinery (Palade, 1953; Frey and Mannella, 2000; Strauss et al., 2008). Hence, the detachment of cristae from the inner boundary membrane likely impacts on the bioenergetic state of mitochondria and may thus

be detrimental in particular for cells with high energy demand, like muscle cells or neurons. MINOS defects have indeed been found to impair respiration and to partially diminish the electrochemical potential across the inner mitochondrial membrane (Darshi et al., 2011; von der Malsburg et al., 2011; An et al., 2012). A massive loss of crista junctions, as observed in mitofilin/Fcj1 and Mio10/MINOS1 mutants, may have adverse effects on the assembly, integrity and function of OXPHOS (super-) complexes, which are predominantly located in cristae membranes (Gilkerson et al., 2003; Vogel et al., 2006; Wurm and Jakobs, 2006). Detachment of cristae from the inner boundary membrane presumably leads to a separation of respiratory chain complexes from the protein import machineries and thus from the supply with substitute components required for the replacement of damaged subunits. Importantly, the OXPHOS machinery is one of the main sources of reactive oxygen species (ROS) in the cell. ROS have been suggested to be a leading cause for mitochondrial DNA mutations and contribute to the process of aging (reviewed in Balaban et al., 2005). The level of ROS production is particularly high under conditions where components of the OXPHOS machinery are impaired. Notably, depletion of mitofilin has been found to be accompanied by an accumulation of mitochondrial DNA mutations (Rossi et al., 2009), which could be related to an aberrant inner membrane morphology.

Alterations of mitofilin have been described in the context of several different pathologies (Table 2). (i) In human fetal Down syndrome brain, mitofilin levels have been found to be decreased by approximately 50% (Bernert et al., 2002; Myung et al., 2003). (ii) Base pair substitutions leading to alterations in the amino acid sequence of mitofilin have been detected in an animal model for epilepsy (Omori et al., 2002). In a further epilepsy model system, oxidatively damaged mitofilin molecules have been observed (Furukawa et al., 2011). (iii) Mitofilin levels were decreased in heart muscle mitochondria in a mouse model for diabetic cardiomyopathy (Baseler et al., 2011). (iv) Alterations of mitofilin have been described in different animal models for neurodegeneration (Wishart et al., 2007; Wang et al., 2008; McDonald et al., 2009). (v) Oxidative damage as well as altered mitofilin levels have been found in model systems of Parkinson's disease (van Laar et al., 2007, 2009; Burté et al., 2011). In summary, mitofilin is affected in a considerable number of pathologies connected to brain development, function and degeneration, as well as in cardiomyopathy. Future studies will have to unravel which of these effects play a primary role in the pathogenesis of the diseases and which are secondary consequences.

Human pathologies associated with MINOS alterations				
Human disease	Alterations in MINOS		Sources	References
Epilepsy	Amino acid substitutions in mitofilin	Animal model systems		<i>Omori et al., 2002</i>
	Oxidative damage of mitofilin			<i>Furukawa et al., 2011</i>
Down syndrome	Mitofilin levels decreased	Human patient material (fetal brain)		<i>Bernert et al., 2002; Myung et al., 2003</i>
Parkinson's disease	Mitofilin levels decreased; Mitofilin covalently modified	Cellular and organellar model systems		<i>Van Laar et al., 2007; Van Laar et al., 2009; Burté et al., 2011</i>
Neurodegeneration	Alterations of mitofilin levels and electrophoretic mobility	Animal model systems		<i>Wishart et al., 2007; Wang et al., 2008; McDonald et al., 2009</i>
Amyloidosis	Mitofilin and CHCHD3 sequestered into amyloid fibrills	Cellular model system		<i>Olzscha et al., 2011</i>
Cancer	Mitofilin and CHCHD6 levels decreased in response to anticancer therapy	Cellular model systems		<i>Magi et al., 2004; An et al., 2012</i>
Diabetic cardiomyopathy	Mitofilin levels decreased	Animal model system		<i>Baseler et al., 2011</i>
MINOS interactors involved in human diseases and aging				
Protein name	MINOS subunits	Associated with	Assigned functions	References
DISC1	Mitofilin CHCHD6	Schizophrenia; Bipolar disorder; Depression	Maintenance of synapses	<i>Camargo et al., 2007; Park et al., 2010; An et al., 2012</i>
HSPA9	Mitofilin CHCHD3 MINOS1	Myelodysplastic syndrome; Parkinson's disease	Chaperone	<i>Xie et al., 2007; Alkhaja et al., 2012</i>
OPA1	Mitofilin CHCHD3	Dominant optic atrophy	Mitochondrial fusion GTPase	<i>Darshi et al., 2011</i>
PARP-1	Mitofilin	Aging	DNA repair	<i>Rossi et al., 2009</i>
PINK1	Mitofilin	Parkinson's disease	Mitochondrial serine/threonine kinase	<i>Weihsen et al., 2009</i>
PNKP	Mitofilin	MCSZ (neurological disorder)	DNA repair	<i>Tahbaz et al., 2012</i>

**Table 2** Multiple links between MINOS and human diseases.

MINOS subunits have been found altered under pathological conditions, and multiple disease-related proteins were suggested to physically interact with MINOS components.

Additionally, MINOS subunits have been found to interact with a number of proteins that are known modulators of human diseases (Table 2). (i) Several studies reported

on an interaction of the MINOS components mitofilin and CHCHD6 with the disrupted in schizophrenia 1 (DISC1) protein (Camargo et al., 2007; Park et al., 2010; An et al.,

2012). Mutations in DISC1 are linked to psychiatric disorders, including schizophrenia, bipolar disorders and major depression (Millar et al., 2000; Brandon and Sawa, 2011). (ii) The serin/threonine kinase PINK1, which is mutated in familial early-onset forms of Parkinson's disease, has been found in a complex with mitofilin (Weihsen et al., 2009). (iii) Mitofilin has also been reported to interact with two DNA repair enzymes involved in development and aging, polynucleotide kinase/phosphatase (PNKP) and Poly(ADP-ribose)polymerase-1 (PARP-1) (Rossi et al., 2009; Tahbaz et al., 2012). It is unknown what the functional significance of these interactions may be. In a recent study by Olzscha et al. (2011), model proteins designed to form fibrillar aggregates similar to those involved in the etiology of Alzheimer's, Parkinson's and Huntington's disease were expressed in cultured cells to identify endogenous proteins prone to co-precipitate with amyloid-like aggregates. Fibrils were found to interact predominantly with multifunctional proteins that associate with many different partners and may function as scaffold-like devices. Seven mitochondrial proteins were found significantly enriched in these fibrils, among them the MINOS components mitofilin and CHCHD3. Thus, MINOS subunits appear to have a tendency to engage in promiscuous protein-protein interactions. Sequestration of MINOS subunits and other proteins with distinct functions into aberrant interactions may contribute to pathological processes, potentially linking cellular stress and abnormal mitochondrial morphology.

## Conclusions

The typical ultrastructure of mitochondria is a prerequisite for mitochondrial function, which in turn is of crucial

importance for the fitness of cells, tissues and organisms. The recently discovered MINOS complex is a key player in the organization of mitochondrial membrane architecture in different organisms from yeast to humans. Gradual destabilization of MINOS correlates with concomitant loss of crista junctions, which indicates that the integrity of MINOS is required for the formation and/or maintenance of these structures. Moreover, MINOS is involved in the communication between the two mitochondrial membranes and forms contact site complexes through physical interaction with several proteins of the outer membrane. It is remarkable that the same molecular machinery appears to be a key player in the formation of both, crista junctions and inner membrane-outer membrane contact sites, two structural features of mitochondria that have puzzled researchers for several decades. Insights into the molecular mechanisms underlying the multiple functions of MINOS will have a major impact on our understanding of mitochondrial architecture and function and ultimately on the elucidation of pathological processes associated with mitochondrial alterations in human diseases.

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