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Biochimica et Biophysica Acta





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# Cardiolipin and mitochondrial cristae organization

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### ARTICLE INFO

Article history Received 23 December 2016 Received in revised form 3 March 2017 Accepted 18 March 2017 Available online 20 March 2017

Keywords: Cardiolipin Mitochondria Cristae Membrane curvature Non-bilaver lipid Electron transport chain Phospholipid

# ABSTRACT

A fundamental question in cell biology, under investigation for over six decades, is the structural organization of mitochondrial cristae. Long known to harbor electron transport chain proteins, crista membrane integrity is key to establishment of the proton gradient that drives oxidative phosphorylation. Visualization of cristae morphology by electron microscopy/tomography has provided evidence that cristae are tube-like extensions of the mitochondrial inner membrane (IM) that project into the matrix space. Reconciling ultrastructural data with the lipid composition of the IM provides support for a continuously curved cylindrical bilayer capped by a dome-shaped tip. Strain imposed by the degree of curvature is relieved by an asymmetric distribution of phospholipids in monolayer leaflets that comprise cristae membranes. The signature mitochondrial lipid, cardiolipin (~18% of IM phospholipid mass), and phosphatidylethanolamine (34%) segregate to the negatively curved monolayer leaflet facing the crista lumen while the opposing, positively curved, matrix-facing monolayer leaflet contains predominantly phosphatidylcholine. Associated with cristae are numerous proteins that function in distinctive ways to establish and/or maintain their lipid repertoire and structural integrity. By combining unique lipid components with a set of protein modulators, crista membranes adopt and maintain their characteristic morphological and functional properties. Once established, cristae ultrastructure has a direct impact on oxidative phosphorylation, apoptosis, fusion/fission as well as diseases of compromised energy metabolism.

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Abbreviations: OM, outer membrane; IM, inner membrane; IBM, inner boundary membrane; EM, electron microscopy; ETC, electron transport chain; MICOS, mitochondrial contact site and cristae organizing system; PE, phosphatidylethanolamine; PS, phosphatidylserine; Opa1, optic atrophy 1; PHB, prohibitin; TAZ, tafazzin gene; DNAJC19, DNA-J homolog subfamily C member 19; FAD, flavin adenine dinucleotide; TCA, tricarboxylic acid; NAD, nicotinamide adenine dinucleotide; CoA, Coenzyme A; SPFH, stomatin/prohibitin/flotillin/HflK; WT, wild type.

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# 1. Introduction: cristae morphology and ultrastructure

Mitochondria are double membrane, reticulated organelles that function in cellular respiration, signaling, cell growth and apoptosis [1]. Both the size and number of mitochondria per cell are variable and, generally, are reflective of the organism, tissue and cell type (Fig. 1). Whereas erythrocytes have none, a single liver cell may contain as many as two thousand mitochondria [2,3]. The outer membrane (OM) surrounds the organelle and houses proteins that regulate ion, lipid and protein import. Separated by the inter-membrane space, the inner membrane (IM) aligns adjacent to the OM. A distinguishing feature of the IM is the presence of numerous villus-like invaginations termed cristae (singular = crista). As such, the IM can be divided into two distinct regions, the inner boundary membrane (IBM) and cristae. Given



**Fig. 1.** Ultrastructure and morphology of mitochondria. A) Image of cell interior depicting mitochondria (gold) of different shapes and sizes. Cristae are seen as digitiform extensions from the periphery into the matrix space. The cell nucleus is shown in red (from [74] with permission). B) Transmission electron micrograph (longitudinal section) of a bat pancreas mitochondrion. Cristae fine structure is depicted, revealing closely apposed, roughly parallel, membrane extensions (see black arrow; image by Keith R. Porter). C) Electron tomography image of cristae from a chick cerebellum mitochondrion. This computer-derived model was generated from segmented 3D tomograms. Cristae are depicted in yellow and the IBM in blue (from [6] with permission).

the presence of multiple cristae in each mitochondrion (Fig. 1), the surface area of the IM (IBM plus cristae) is up to 4 times greater than that of the OM. Originally described as membrane folds or septa (e.g. the "baffle" model commonly depicted in textbooks [4]), technical advances in ultrastructure analysis of mitochondria by electron microscopy (EM) and high voltage EM tomography have led to the current view of cristae as tubules [5-8]. Despite this consensus, alternate structures have been described including interconnected tubules, tubulo-vesicular structures, lamellar cisternae or flask-shaped buds [9–11]. Considerable variability in cristae morphology and ultrastructure exists, reflecting species/tissue differences, the bioenergetic state of the organelle, osmotic environment and/or preparation related effects. It is also clear that protein-mediated modulation of cristae morphology, including expansion and contraction, are physiologically relevant processes [12-14]. One of earliest examples of mitochondrial dynamics is the transition from an "orthodox" to "condensed" state reported by Hackenbrock [15]. In this classic study, EM analysis of isolated mitochondria revealed that cristae undergo dramatic shape changes in response to alterations in metabolic state.

To this day, understanding the role(s) played by distinct lipids and protein modulators of crista biogenesis, organization, structure and dynamics is of great interest. With regard to the role of lipids in cristae formation, Khalifat et al. [16,17] employed giant unilamellar vesicles (GUV) in studies of the effect of local pH change on membrane deformation. Remarkably, cardiolipin containing GUV were induced to form narrow tubular extensions upon micropipette manipulation of the local pH. The resulting cristae-like invaginations were dynamic, progressing or regressing as a function of pH modulation. The authors concluded that cardiolipin plays a crucial role in the formation of this cristae-like tubular morphology in GUV membranes and this effect may be relevant to cristae biogenesis in mitochondria. Increasing the potential physiological relevance of GUV tubule formation, Ban et al. [18] reported that the dynamin-like GTPase, Opa1-S, binds cardiolipin containing liposomes and promotes protrusion of tubular membrane extensions (50-100 nm in diameter). Thus, it is conceivable that, in the physiological setting of the IM, cardiolipin interaction with Opa1-S induces membrane shape changes that lead to the biogenesis of tubular cristae.

### 2. Structure and function of tubular cristae

As depicted in Fig. 1, panel C, tubular cristae protrude into, and are bathed in, the aqueous matrix space of the mitochondrion. This compartment is rich in metabolic enzymes whose activity feeds reducing equivalents into the electron transport chain (ETC; Fig. 2). Stored or imported fuel molecules, including fatty acids, glucose (pyruvate) and amino acids, are metabolized to acetyl CoA, which is subsequently oxidized via the TCA cycle. For each acetyl CoA that enters this cycle, two CO<sub>2</sub>, three NADH and one FADH<sub>2</sub> are generated. NADH is a substrate for Complex I while FADH<sub>2</sub> is a substrate for Complex II of the crista membrane-embedded ETC. In essence, these redox cofactors connect metabolic activity in the matrix to electron transfer reactions within the crista membrane. Passage of electrons from low to high standard reduction potential drives translocation of protons into the crista lumen. Cytochrome c oxidase (Complex IV) receives electrons from Complex III (Coenzyme Q - cytochrome c reductase) and transfers them to oxygen to produce water in reactions that are coupled to proton translocation into the crista lumen. Key to preventing nonproductive dissipation of this proton gradient is a well-defined and maintained crista membrane that completely envelops the lumen space. As the concentration of protons in the crista lumen increases, this proton-motive force is employed as a "substrate" for F<sub>1</sub>F<sub>0</sub> ATPase (Complex V), effectively coupling proton transit from the crista lumen into the matrix space to ADP phosphorylation. This series of events is the raison d'être for cristae and proper functioning of these inter-related processes requires that crista membrane integrity be maintained and that proton diffusion at the crista junction does not occur. As described below, cristae possess



**Fig. 2.** Diagram of mitochondrial energy metabolism and cristae organization. Fatty acid β-oxidation, glycolysis and amino acid metabolism generate acetyl CoA that fuels the TCA cycle, producing NADH and FADH<sub>2</sub>. These reduced cofactors are oxidized by Complexes I and II, respectively, of the ETC. The transmembrane ETC complexes are depicted as various shaped and colored objects within each crista. The outermost cristae in the diagram shows the exterior, matrix facing membrane surface while the two cristae toward the center of the diagram show a cut-away view, revealing their interior lumen space. As NADH and FADH<sub>2</sub> are oxidized to NAD<sup>+</sup> and FAD<sup>+</sup>, free energy from electron transfer is used to pump H<sup>+</sup> into the crista lumen, generating an electrochemical gradient across the crista membrane. This gradient is the driving force for ATP synthesis via Complex V (ATP synthase; depicted as yellow objects).

characteristic structural and organizational features that create an optimal environment for efficient, continuous coupling of metabolic activity, electron flux, proton translocation and ADP phosphorylation. On closer inspection, each crista tubule can be divided into three distinct regions.

# 2.1. Crista junction

The crista junction is the site where a given crista extends inward from the IBM [19]. The diameter at the mouth of a crista junction has been estimated to be ~28 nm [7]. A prominent feature of the crista junction is a  $\sim 90^{\circ}$  bend in the IBM. At this site opposing leaflets of the bilayer experience significant positive (inner leaflet) and negative (outer leaflet) curvature. Phospholipids capable of residing in an elastically strained membrane environment are key to maintenance of membrane integrity at these sites. At the same time, an essential role is played by the mitochondrial contact site and cristae organizing system (MICOS), a sophisticated protein-based machinery that function in the assembly and maintenance of crista junctions [11,20-23]. Moreover, the MICOS creates a diffusion/transit barrier for proteins and metabolites, thereby separating the inner crista space (i.e. the crista lumen) from the intermembrane space. In a similar manner, it is likely that lipid trafficking between cristae membranes and the IBM is restricted at the crista junction, allowing these physically connected membrane segments to possess distinct lipid compositions [24].

### 2.2. Crista stalk

Depending on its length, the digitiform stalk segment comprises up to 95% of the surface area of a given crista. Extending from the crista junction into the matrix space, the stalk segment harbors ETC supramolecular complexes and the  $F_1F_0$  ATPase. Observed in EM images as roughly parallel membrane extensions (Fig. 1B) that separate the crista

lumen from the matrix space, EM tomography analysis [7] reveals a largely tubular ultrastructure (Fig. 1C). Maintenance of crista stalk membrane bilayer integrity serves to stabilize embedded ETC complexes and limits diffusion of protons that accumulate in the crista lumen as a result of ETC activity. These structural and organizational properties are anticipated to enhance aerobic ATP production efficiency.

# 2.3. Crista tip

Capping the stalk segment of each crista is the tip region. Consistent with a tubular morphology of intact crista, the tip segment serves as a dome-shaped cap that seals the membrane, forming a barrier between the crista lumen and matrix space environments. The crista tip region has alternatively been depicted as a "rim" that connects closely apposed parallel lamellar sheets that may be present [11].

A key protein component of cristae often localized to the tip region is the ATP synthase complex, also termed F<sub>1</sub>F<sub>0</sub> ATPase. This large protein complex (~600 kDa) uses the proton electrochemical gradient generated by ETC activity to catalyze ATP synthesis from ADP and P<sub>i</sub>. Notwithstanding its role in ATP synthesis, F1F0-ATPase has been reported to influence cristae morphology. In many systems, this complex adopts dimeric or oligomeric structures that align in a row at the tip (and stalk region) of cristae membranes [11,25,26]. Electron cryotomography studies have provided evidence that "ribbons of dimers" form in the IM of rat and bovine mitochondria. Given that dimer rows are found in curved regions of cristae membranes, they either localize to, or induce formation of, membrane curvature. Evidence that dimer rows influence cristae morphology has been obtained in deletion studies. When dimerization-specific  $F_1F_0$ -ATPase subunits *e* or *g* (or the first helix of subunit b) were deleted, tubular cristae morphology was replaced by an aberrant, "onion-like" morphology [27]. Despite these results, it remains possible that lipid components of cristae membranes, specifically cardiolipin, create an environment that induces ATP synthase dimerization. Consistent with this, Acehan et al. [28] reported that cardiolipin deficiency disrupts the long-range assembly pattern of ATP synthase in *Drosophila* flight-muscle mitochondria. These authors examined ATP synthase organization in mitochondria of wild type (WT) versus cardiolipin synthase deficient flies by cryoelectron tomography. Compared to WT mitochondria, cardiolipin synthase deficient mitochondria (with nearly complete loss of cardiolipin) manifested less extended dimer rows and more "scatter" in dimer orientation. These data suggest the presence of cardiolipin promotes a ribbon-like assembly of ATP synthase dimers through effects on the organization and morphology of crista membranes.

### 3. Phospholipid asymmetry in cristae membranes

Any model of crista organization derived from ultrastructural analysis must reconcile with the phospholipid composition of the IM. A noteworthy and distinguishing component of the IM is cardiolipin (18% of phospholipid mass; [29]. Unlike other glycerophospholipids, cardiolipin possesses two phosphatidyl moieties that share a glycerol head group. The combination of a compact anionic polar head group and four esterified fatty acyl chains results in a distinctly cone-shaped molecule [30]. Based on its properties, cardiolipin is classified as a "non-bilayer" phospholipid [31,32]. Likewise, phosphatidylethanolamine (PE), which comprises 34% of IM phospholipid mass, is also a cone-shaped, non-bilayer phospholipid. How does a membrane that contains ~50% non-bilayer phospholipids maintain a bilayer structure? Most likely, a planar bilayer could not accommodate such high percentages of PE and cardiolipin. At the same time, owing to their cone-shaped structures, PE and cardiolipin are "tailor made" to reside in, and stabilize, a curved membrane by segregating into the negatively curved monolayer leaflet.

A curious feature of cardiolipin is its virtual absence from nearly all other cell/organelle membranes in nature. One exception to this general rule is bacteria. For example, the cytoplasmic membrane of *Escherichia coli* contains ~5% cardiolipin [33]. The polar ends of *E. coli* display a high degree of curvature and studies indicate cardiolipin segregates into the negatively curved inner leaflet of the cytoplasmic membrane at the polar ends of this rod-shaped prokaryote [34,35]. Segregation of cardiolipin in this manner effectively relaxes elastic strain that would otherwise exist in this region of high membrane curvature. Given its role in *E. coli* membranes, it is reasonable to posit that cardiolipin in

the IM of mitochondria serves a similar function. At the same time, it is recognized that considerable variability exists among prokaryotes with respect to cardiolipin content. Given that this variability extends to bacterial shape (e.g. rods, cocci, spiral) and overall lipid composition, more work is required to understand the precise role(s) of cardiolipin in different bacterial species. A common feature relating *E. coli* to bacteria in general, however, is the proposed role of cardiolipin in binary fission as a component of membrane septa formation during cell division [36–38].

Cardiolipin and PE are known to generate negative curvature elastic stress in bilayers [33,39]. Based on their relative abundance in the IM (~50% of the phospholipid mass), it is evident that sufficient "negative curvature phospholipids" exist in cristae to stabilize a bilayer that is 100% curved. This seemingly high proportion of curved membrane is in agreement with EM and tomography results that depict cristae as tubular structures (Fig. 1). If so, then it may be anticipated that the monolayer leaflet facing the crista lumen is highly enriched in PE and cardiolipin while the opposing, positively curved matrix facing monolayer leaflet, contains predominantly phosphatidylcholine (~80%), with lesser amounts of phosphatidylserine (PS) and phosphatidylinositol (Fig. 3). This asymmetric phospholipid distribution will confer stability to a continuously curved crista membrane, providing an explanation for the high proportion of non-bilayer phospholipids in the IM. Notwithstanding dynamic changes in ultrastructure and morphology, the diameter of a crista has been estimated to be ~35 nm. Assuming the width of a bilayer to be ~4 nm, the internal diameter of a crista tubule is ~27 nm. The internal aqueous space (i.e. the crista lumen) is bounded on all sides by a protein-rich membrane whose PE- and cardiolipin-containing inner monolayer leaflet is continuously negatively curved (radius of curvature ~15 nm).

Ultrastructure studies reveal the length of cristae to be variable, some as long as 1 µm while others are much shorter. Regardless of their length, however, the percentage of curved membrane will not change since the dome-shaped crista tip membrane is similarly curved, positive on the matrix side and negative on the crista lumen side. Likewise, the membrane segment corresponding to the crista junction also experiences significant curvature, although in this case it is negatively curved on the matrix side and positively curved on the inter-membrane space side. The model of a crista that emerges is an elongated, cylinder-shaped bilaver that employs cardiolipin and PE to relieve strain in the



Fig. 3. Phospholipid asymmetry in cristae membranes. To stabilize a bilayer membrane with an ~15 nm radius of curvature, phosphatidylcholine (PC), phosphatidylinositol (PI) and PS segregate into the positively curved leaflet while cardiolipin (CL) and PE partition into the apposing negatively curved leaflet. Phospholipid segregation in this manner decreases torsional strain that would otherwise exist in a highly curved bilayer. Not shown are the abundant protein components of cristae membranes, including ETC complexes. The phospholipid composition of the IM of rat liver mitochondria [29] is presented.

negatively curved monolayer leaflet facing the crista lumen. An important feature related to crista membrane phospholipid asymmetry is the fact that PE and cardiolipin are produced on site [32]. For example, PE is generated in one step by decarboxylation of PS. Preformed PS is imported from the endoplasmic reticulum to the matrix face of the IM, where PS decarboxylase is located. Following this reaction, newly formed PE segregates to the lumen facing monolayer leaflet of the crista membrane, possibly driven by a curvature-mediated mechanism [40]. Support for the concept that PE and cardiolipin play indispensable roles in the IM has emerged from studies designed to alter their content. When PE levels are reduced by genetic disruption of PS decarboxylase, cristae morphology and oxidative phosphorylation are impaired [41].

In a similar manner, cardiolipin is generated within the IM following import of the precursor lipids, phosphatidylglycerol and CDP-diacylglycerol [32]. Disruption of cardiolipin biosynthesis results in altered cristae morphology [42], decreased ETC supercomplex formation [43], increased proton leak [44] and impaired membrane potential [45]. In cardiac and skeletal muscle mitochondria, cardiolipin undergoes a maturation cycle that involves enzyme-catalyzed acyl chain remodeling (see below). Attesting to the importance of this process, loss of remodeling activity has a major impact on IM morphology and respiration [42, 46,47]. Thus, it appears that the function(s) of PE and cardiolipin in the IM cannot be compensated by other phospholipids.

# 4. Protein modulators of crista membrane structure and cardiolipin content

In addition to its structural role as a component of cristae membranes, cardiolipin directly interacts with ETC proteins and, moreover, is required for their optimal activity in vitro [48,49]. Given the unique phospholipid composition of cristae, their elongated cylindrical shape and susceptibility to disruption by external forces, it is not unexpected that proteins play an essential role in the biogenesis and maintenance of cristae. Just as the MICOS is necessary for proper assembly of crista junctions, specialized proteins have been identified that modulate crista morphology, lipid composition and fusion/fission events. As described above, this includes proteins that function in phospholipid biosynthesis and transport [32] as well as protein components of the MICOS [50]. Below we describe cristae membrane-associated proteins with an established connection to cardiolipin. In many cases, overexpression or deletion of these proteins alters cardiolipin content and composition and/or induces morphological alteration of cristae membrane structure.

### 4.1. Opa1

Opa1 is a dynamin-like GTPase that functions in crista membrane fusion [51], modulation of crista structure [12] and crista remodeling during apoptosis [52]. Mutations in OPA1 cause dominant optic atrophy, a disorder characterized by altered mitochondrial morphology and defective energy metabolism [53]. Opa1 exists in either a long (L) or short (S) form. Opa1-L is bound to the IM via an N-terminal hydrophobic region with the bulk of the protein protruding into the crista lumen/inter-membrane space. Opa1-S is released from Opa1-L as a soluble protein following proteolysis by the metalloenodpeptidase, OMA1 [54] and the relative abundance of the L and S forms of Opa1 influence subsequent membrane fusion/ fission events. Interestingly, the yeast homolog of Opa1, Mgm1, forms higher order structures that promote membrane fusion in a cardiolipin-dependent manner [55]. Furthermore, Opa1 responds to the bioenergetic state of mitochondria, forming oligomers that tighten cristae when substrates are available. Conceivably, Opa1 mediates dynamic changes in crista morphology that correlate with the metabolic state of the organelle [12].

### 4.2. Tafazzin

The X-linked tafazzin (TAZ) gene encodes a phospholipid transacylase [56,57]. In humans, alternative splicing of TAZ mRNA yields distinct protein products with variable levels of transacylase activity. The splice form with maximal activity, and the ability to complement a TAZ deletion mutant yeast strain, is ∆exon 5 tafazzin [58]. The N-terminal region of tafazzin contains a stretch of hydrophobic residues that likely functions as a membrane interaction site. Tafazzin enzyme activity has been well studied in vitro, most often using a glycerophospholipid (acyl donor)/lysophospholipid (acyl acceptor) pair. Under these conditions tafazzin catalyzes acyl chain exchange and it is considered that repeated reactions of this type are responsible for cardiolipin molecular species homogenization (~90% tetralinoleoylcardiolipin) in skeletal muscle and cardiac tissue mitochondria. It is interesting to note that, despite the expression of TAZ mRNA, other tissues (e.g. brain) do not display the same the same degree of cardiolipin acyl chain homogeneity [59].

It has been suggested that, in tissues with high oxidative capacity, tafazzin-mediated acyl chain remodeling promotes optimal crista membrane lipid packing. At the same time, in vitro transacylase assays indicate tafazzin is not specific for linoleoyl chains nor does it display a substrate preference for cardiolipin versus other glycerophospholipids [60]. This aspect of tafazzin catalytic activity has led to the concept that substrate availability or presentation, rather than intrinsic acyl group specificity, dictates the ultimate thermodynamic equilibrium achieved. Schlame et al. [61] reported that tafazzin-mediated transacylase activity is lipid phase dependent, with higher activity occurring in non-bilayer phases. Based on this, a concept referred to as "thermodynamic remodeling" was proposed wherein the acyl chain specificity of tafazzin-dependent transacylation reactions is imparted by the physical properties of the "membrane". An interesting concept, consistent with a high proportion of cardiolipin in the crista membrane monolayer leaflet facing the crista lumen, is that the intrinsic radius of curvature, a high membrane protein content and ongoing electron transfer reactions increase susceptibility to disturbances in lipid-packing order. As membrane lipid damage occurs, non-bilayer "pockets" may appear in the context of an otherwise intact bilayer membrane. In this case, ensuing tafazzin-mediated acyl chain remodeling may function to re-establish optimal lipid packing order, thereby restoring membrane integrity. In Barth syndrome, mutations in TAZ lead to specific changes in cardiolipin content and composition, including decreased amounts of cardiolipin, greater cardiolipin acyl chain heterogeneity and increased levels of monolysocardiolipin [62]. Studies have documented that these changes positively correlate with profound alterations in cristae morphology and compromised aerobic energy metabolism [46,63].

#### 4.3. MICOS proteins

Collectively, MICOS proteins are involved in biogenesis and maintenance of cristae junctions as well as tethering the inner and outer membranes. For example, the auxiliary MICOS interacting protein, Aim24, regulates mitochondrial architecture, morphology as well as the protein and lipid composition of cristae [64]. Aim24 localizes to the mitochondrial IM and is required for the integrity of the MICOS complex. Studies with yeast Aim24 deletion mutants revealed that this gene is required for respiratory growth, stability of ETC supercomplexes and mitochondrial ultrastructure [64,65]. Whereas mitochondria from cells lacking Aim24 do not manifest an altered cardiolipin composition, when Aim24 deletion is combined with subtle modifications (appending a His-Tag sequence) to other MICOS proteins (MIC12 or MIC26), an altered cardiolipin acyl chain pattern is observed, with a shift toward longer, more saturated acyl chains. Consistent with its known role in cardiolipin acyl chain remodeling, mutant cells had reduced levels of the tafazzin transacylase. Together, these data establish a link between

ETC supercomplex formation and tafazzin-dependent cardiolipin acyl chain remodeling. Thus, despite the fact that Aim24 is not directly involved in membrane phospholipid metabolism, it affects the spatial organization of mitochondrial proteins and cristae membrane lipid composition.

MIC26, formerly known as APOO, localizes to the IM and serves as a component of the MICOS. Co-immunoprecipitation studies [20] revealed that MIC26 interacts with other MICOS proteins including MIC60, MIC27 and MIC10. One of these proteins, MIC27 (formerly known as APOOL), is related to MIC26 and has been shown to bind cardiolipin [66]. MIC26 and MIC27 reciprocally influence levels of one another and upon overexpression of either MIC26 or MIC27, a corresponding increase in levels of MIC10 and tafazzin were observed. Likewise, when levels of MIC26 or MIC27 are decreased, reciprocal regulation was retained while MIC10 and tafazzin levels were reduced. Depletion of MIC26 or MIC27 also results in impairment of mitochondrial respiration, consistent with an ability to influence mitochondrial ultrastructure and the number of cristae junctions. The extent to which the function(s) of MIC26 and MIC27 overlap, and the degree to which one is able to compensate for the other, are yet to be elucidated. Likewise, it is currently unknown whether fluctuation in tafazzin abundance upon manipulation of MIC26 or MIC27 levels affects the content or composition of cardiolipin. Recently, however, Friedman et al. [21] reported that MIC27 binding to a distinct MICOS subcomplex is cardiolipin dependent, suggesting cardiolipin is required for MIC27 localization to cristae junctions.

### 4.4. Prohibitins and stomatin like protein-2

Proteins of the stomatin/prohibitin/flotillin/HflK (SPFH) family interact with membranes, serving a scaffold function. In eukaryotes, the prohibitins (PHBs) function as scaffolds in detergent resistant membrane microdomains [67]. PHBs exist as two closely related proteins (PHB1 and PHB2) that localize to the IM. Interestingly, PHBs assemble into ring-like structures comprised of 16–20 alternating PHB1/PHB2 subunits [68]. While the precise function of PHB ring structures has vet to be established, a chaperone function for respiratory chain proteins or scaffold that stabilizes crista have been proposed [68]. It is noteworthy that the diameter of an assembled PHB ring (~25 nm) corresponds well with the estimated interior diameter of a tubular crista lumen. Thus, it is conceivable that PHB ring structures provide a framework that a) fixes the radius of curvature of crista membranes and/or b) stabilizes the structure of crista tubules (Fig. 4). In this manner, PHB rings may protect against bilayer collapse or coalescence of neighboring cristae. Consistent with such a role, in the absence of one or both PHB subunits, crista membrane morphology is dramatically altered [68]. Another potential function of assembled PHB ring structures could be to restrict lipid movement/diffusion, thereby promoting establishment/ maintenance of microdomains within the IM [67]. Intriguingly, silencing PHB expression leads to characteristic changes in the acyl chain composition of cardiolipin as well as the appearance of monolysocardiolipin [69]. These data imply that tafazzin-mediated transacylase activity may be dependent upon the presence of an intact PHB ring structure.

Another member of the SPFH protein family present in the IM is stomatin-like protein 2 (SLP-2). Interestingly, SLP-2 has been shown to bind cardiolipin and interact with PHBs [70,71]. Consistent with this, *slp-2* deletion correlates with decreased levels of cardiolipin and PHBs in mitochondrial detergent-insoluble membranes. Furthermore, abnormal compartmentalization of cardiolipin in the absence of SLP-2 correlates with decreased ETC supercomplex formation. Thus, it has been proposed that SLP-2 functions to stabilize IM structure and/or serve as a platform for supercomplex assembly. The role of SLP-2 in formation of cardiolipin-rich microdomains likely takes advantage of its cardiolipin binding activity as well as its ability to recruit PHBs [68,72].

### 4.5. DNAJC19

DNAJC19 is an apparent mitochondrial chaperone protein that gained attention in studies of the Canadian Dariusleut Hutterite community [73]. Autosomal recessive mutations in DNAJC19 were identified



Fig. 4. PHB ring orientation in a crista membrane. A) Electron micrograph of a mitochondrion. A portion of a single crista stalk region (yellow box) has been schematically expanded (B) to indicate a continuously curved, cylindrical bilayer membrane that envelops the crista lumen space. In (C) an assembled, membrane-associated, PHB ring circumscribes the interior of the crista membrane, providing a structural scaffold and/or diffusion barrier (adapted from Osman et al. [68] with permission).

in subjects from this community diagnosed with a disorder termed Dilated Cardiomyopathy with Ataxia syndrome. Curiously, phenotypic features of this disorder resemble those seen in Barth Syndrome and, recently, Richter-Dennerlein et al. [69] discovered that DNAJC19 interacts with PHB complexes in mitochondria. Although sequence analysis suggests a role in mitochondrial protein import, experimental evidence is consistent with a chaperone function for DNAJC19 in cristae. Upon silencing DNAJC19 mRNA, alterations in cardiolipin acylation and crista morphology, reminiscent of those seen upon PHB silencing or *TAZ* mutation, were noted. How these distinct protein components of the IM influence membrane structure and integrity is not clear at this time but their effects on crista morphology and energy metabolism are likely to be far-reaching.

# 5. Conclusions

Cristae membranes contain a high protein content, as well as significant amounts of non-bilayer phospholipids. In keeping with their coneshaped structures, cardiolipin and PE function to lessen torsional stain in curved bilayers by segregating into the negatively curved monolayer leaflet. Tubular crista morphology serves to minimize crista lumen volume and, thereby, increase the effective concentration of solutes, especially protons, whose passage from the lumen to the matrix space is coupled to ADP phosphorylation through the action of the  $F_1F_0$  ATPase. A wealth of new information is emerging about the function of specialized proteins that modulate cristae dynamics, including establishment and maintenance of crista junctions, fusion, fission, expansion and contraction. While this review describes some of key players with a direct connection to cardiolipin, it is a virtual certainty that numerous additional proteins are involved in the formation and maintenance of cristae.

### **Transparency document**

The Transparency document associated with this article can be found, in the online version.

### Acknowledgements

The authors thank Ms. Maria Carrillo-Hernandez for expert assistance. This work was supported by a grant from the US National Institutes of Health (R37 HL64159). NI was supported by NIH training grant T32 DK061918.

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