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# Reconstitutions of mitochondrial inner membrane remodeling

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ABSTRACT

Biological membranes exhibit function-related shapes, leading to a plethora of complex and beautiful cell and cell organellar morphologies. Most if not all of these structures have evolved for a particular physiological reason. The shapes of these structures are formed by physical forces that operate on membranes. To create particular shaped cells and cell organelles, membranes must undergo deformations which are determined by the structure and elasticity of the membrane and this process is most probable driven by proteins, lipids and/or interplay of both Zimmerberg and Kozlov (2006). Therefore, an important question of current cell biology in conjunction with physics and mathematics is to elucidate the functional cause for these different membrane morphologies as well as how they are formed.

One of the most peculiar membrane shapes is observed in mitochondria. These organelles are surrounded by two membranes and especially the convoluted inner membrane displays a complex ultrastructure. A molecular understanding of how this membrane is shaped is missing to a large extent. Unlike membrane remodeling in classical curvature-dependent processes like clathrin-mediated endocytosis, mitochondria are most likely shaped by integral membrane proteins. Following, we will review the current knowledge of inner mitochondrial membrane architecture and discuss recent findings and advances in understanding the factors that shape this membrane. We will address pending questions especially with regard to the experimentally challenging nature of investigating membrane bending by hydrophobic integral membrane proteins.



## 1. Introduction

Membrane-bound organelles of eukaryotic cells display a wide variety of morphologies (Zimmerberg and Kozlov, 2006). For example, peroxisomes and lysosomes adapt relatively simple spherical shapes, whereas the Golgi apparatus and the endoplasmic reticulum form sophisticated networks consisting of sheet-like, tubular and interconnected membrane structures. Mitochondria also form an extended, ever-changing network in the cell, but as they are enclosed by two membranes the mitochondrial ultra-structure exhibits another level of complexity. The outer membrane (OM) borders mitochondria from the rest of the cell. The inner membrane (IM) is highly folded and consequently occupies a much larger surface than the OM. This allows the accommodation of high concentrations of respiratory chain complexes and of the $F_1F_O$-ATP synthase. These protein complexes are predominantly found within the cristae membranes, invaginations of the inner membrane towards the matrix. Because of the convoluted structure, the IM can be divided into distinct morphological regions displaying various degrees of membrane curvature: the inner boundary membrane (IBM), which is in close proximity to the outer membrane and, at the level of the dimensions of a single protein, can be regarded as flat; the cristae membranes are diverse and depending on the cell type, tissue or energy level of the cell can adopt a variety of different shapes from highly curved tubular to more flat lamellar structures (Zick et al., 2009a); the cristae rims and the cristae junctions (CJs), which are narrow tubular openings connecting IBM and cristae membranes, display high degrees of membrane curvature (Fig. 1). The shape, size and number of cristae membranes is dynamic and variable probably to respond to different energetic demands of the cell. For example mammalian mitochondria from rapidly contracting muscle cells show a higher number of more densely packed cristae membranes than mitochondria from moderately active epididymal epithelium (Fawcett, 1981). Excellent summaries of this topic can be found elsewhere (Frey and Mannella, 2000). In contrast to the shape and size variety of cristae membranes, CJs are rather uniform with average diameters between 10 and 30 nm (Frey and Mannella, 2000; Mannella,

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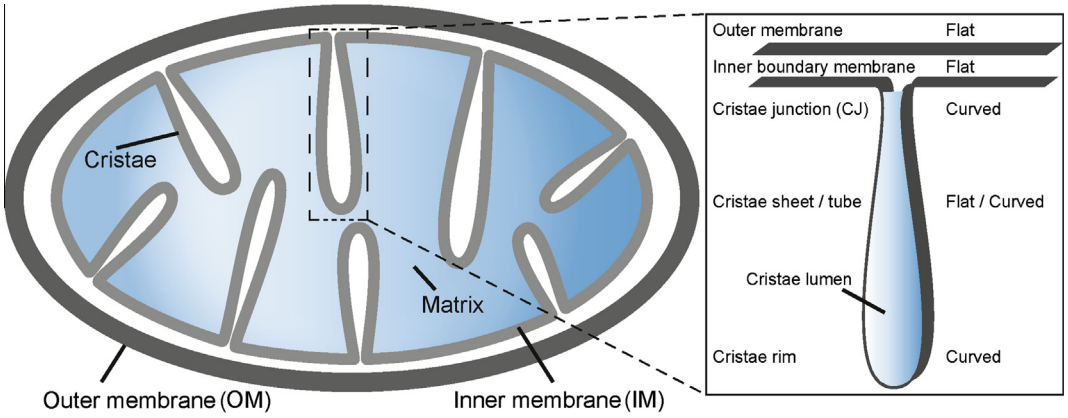


**Fig. 1.** Schematic illustration of mitochondrial ultra-structure. Mitochondria are enclosed by two morphologically and functionally distinct membranes. The outer membrane borders the organelle from the cytosol. Functionally distinct regions that adopt different degrees of membrane curvature (as indicated) compose the inner membrane.

2006; Renken et al., 2002). CJs are believed to act as structural organizers within the IM and therefore have a significant impact on mitochondrial processes. Over the last years accumulating evidence was found for an uneven protein distribution along the IM, giving rise to the model that the IM is divided into regions of specific protein content and specialized function (Mannella et al., 1994; Rabl et al., 2009; Stoldt et al., 2012; Strauss et al., 2008; Vogel et al., 2006; Williams, 2000; Wurm and Jakobs, 2006). CJs are believed to maintain this functional compartmentation of the IM by acting as diffusion barriers (Frey et al., 2002; Mannella et al., 1997; Perkins et al., 1997). The importance of CJs for mitochondrial and cellular physiology is further underlined by their role in the intrinsic apoptotic pathway. CJs participate in the regulation of apoptosis by sequestering the release of pro-apoptotic factors (e.g., cytochrome c) from the cristae lumen to the cytosol (Galluzzi et al., 2012; Pellegrini and Scorrano, 2007; Scorrano et al., 2002). Finally, the second highly curved, structure-bearing element of the IM are the cristae rims. Though structurally well described, their role for physiological processes is poorly understood. One model suggests that the geometry found at cristae rims facilitates effective utilization of the proton gradient across the inner mitochondrial membrane and ultimately ATP production (Strauss et al., 2008).

The peculiar architecture of the IM and in particular the physiological importance of CJ integrity suggests a tight regulation of these structures by proteins and/or lipids. A number of proteins, protein complexes and lipids have been proposed to be involved in cristae membrane biogenesis and in shaping the IM (Neupert, 2012). However, with few exceptions, their direct contribution in membrane curvature formation including possible mechanisms of action remains one of the big unresolved questions of mitochondrial biology. Recent investigations started to shed light on this issue (Barbot et al., 2015; Bohnert et al., 2015; Davies et al., 2012) and it is an exciting time for mitochondrial biology where we are just beginning to understand how this organelle acquires its unique shape.

Whereas the variety of cristae membrane structures is better understood for mammalian mitochondria CJs and cristae rims seem to be rather similar in yeast and mammals. Therefore, not surprisingly, the factors that are implicated in forming or maintaining CJs and cristae rims appear to be well conserved from yeast to mammals. In case of CJ formation the majority of studies have so far been conducted in yeast and we point out where we talk about mammals as a model organism.

Interestingly, most proteins that are believed to be involved in shaping the IM are integral membrane proteins. While membrane bending by peripheral membrane proteins is fairly well understood, a molecular understanding of how integral membrane proteins affect membrane morphology is missing to a large extent. Due to their physico-chemical properties integral membrane proteins are all the more difficult to examine which possess particular challenges for reconstituted *in vitro* investigations using purified proteins and model membranes.

## 2. Shaping of cristae rims

Over the last 15 years strong evidence was found that the $F_1F_O$-ATP synthase is important for determining the physiological structure of cristae membranes. This multi-subunit protein complex is enriched in the highly curved cristae rims, where ATP synthase dimers form rows of higher order oligomers (Allen et al., 1989; Buzhynskyy et al., 2007; Davies et al., 2012; Strauss et al., 2008). Two subunits e and g (Su e/Su g) of the $F_O$ domain have been shown to be important for dimer formation, which is mediated by highly, conserved glycine-rich (GxxxG) motifs (Arnold et al., 1998; Arselin et al., 2003; Saddar and Stuart, 2005; Wagner et al., 2009). Such glycine-rich motifs have been shown to be important for transmembrane helix packing and as such for membrane protein oligomerization (Alavian et al., 2014; Barbot et al., 2015; Bohnert et al., 2015; Demishtein-Zohary et al., 2015; Russ and Engelman, 2000; Vonck et al., 2002). The possible role of ATP synthase dimers in cristae generation was first proposed by Allen (Allen, 1995). The direct link between ATP synthase dimers and cristae biogenesis became obvious in later studies (Paumard et al., 2002) where it was shown that depletion or alterations of the oligomerization subunits Su e, Su g or Su e/Su g inhibited dimer formation. The resulting morphology was characterized by a lack of cristae tips and therefore by abnormal cristae membranes that arranged in concentric, onion-like IM circles (Fig. 2) (Arselin et al., 2003; Bustos and Velours, 2005; Giraud et al., 2002; Paumard et al., 2002; Rabl et al., 2009; Zick et al., 2009a). Thus, ATP synthase dimers are a prerequisite for normal inner mitochondrial membrane morphology maintenance.

Presently, the widely accepted model of cristae tip formation suggests that ATP synthase dimers generate a local positive curvature in the inner mitochondrial membrane towards the matrix. The angle between two ATP synthase monomers enables the dimer to form a rigid arc and thereby bending the membrane (Dudkina et al., 2006, 2005). Results of molecular dynamic simulations are also in favor of this model, underlining that ATP synthase dimers are sufficient to bend lipid bilayer membranes (Davies et al., 2012).

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