JGP Journal of General Physiology

COMMENTARY

Membrane-mediated interaction drives mitochondrial ATPase assembly and cristae formation

Qiang Cui

Because of rapid progress in various imaging and structural biology techniques, it is increasingly recognized that components of cellular membranes self-organize into remarkable structures with rich architectural features. Striking examples include the immunological synapse (Grakoui et al., 1999), the interconnected system of sheets and tubules in the endoplasmic reticulum (Terasaki et al., 2013; Shemesh et al., 2014), and the stack of perforated sheets in the Golgi apparatus (Boal, 2002). Another fascinating case is the mitochondria inner membrane cristae (Alberts et al., 1994), which is a hallmark signature of mitochondria morphology. In this issue of the *Journal of General Physiology*, new research provides insight into how these cristae are formed.

The morphology of mitochondrial cristae membranes are illustrated schematically in Fig. 1. Cryotomography studies have established that respiratory chain proton pumps (e.g., Complex I) are arranged on the flat regions of the cristae membranes, whereas F_0F_1-ATP synthase molecules form dimer rows along cristae edges (Davies et al., 2011). It was proposed that the highly curved nature of cristae increases the inner mitochondrial membrane surface to accommodate a large number of respiratory chain complexes and hence that this morphology is essential for the high energy output of mitochondria. The spatial distribution of proton pumps and ATP synthases, which are proton sinks, was proposed to support a locally increased proton concentration gradient (however, see the discussion of Rieger et al., 2014) and thus ensure a high efficiency of ATP synthesis. The unique morphology of the cristae and distribution of ATP synthase along them beg the following questions: What drives the formation of the highly curved cristae and the row of ATP synthase dimers? Which forms first, and why are the dimers localized to the cristae edges? How essential is protein–protein interaction to the assembly of ATP synthase dimers? The computational analysis conducted by Anselmi et al. in this issue has provided important insights into these questions, suggesting that the formation of cristae and assembly of ATP synthase dimers are coupled processes that are driven largely by membrane elasticity, whereas specific dimer–dimer interactions likely play a minor role.

It has long been recognized that membrane elasticity plays a major role in influencing the association and properties of membrane proteins. Using gramicidin A (gA) as model systems, the pioneering work of Huang (1986) and Andersen and Koeppe (2007) has demonstrated that the assembly and lifetime of gA dimers can be tuned by modulating the degree of hydrophobic mismatch between the gA dimer and surrounding lipids. Kim et al. (1998), Weikl et al. (1998), and Chou et al. (2001) have thoroughly analyzed the thermodynamics of protein–protein interactions mediated by membrane elasticity using a continuum mechanics treatment of the membrane. These analyses highlight the importance of protein shape and nonpairwise contributions; with an isotropically bent membrane, for example, the pairwise interaction between two proteins (or "membrane inclusions") was predicted to be repulsive. These studies formed the basis of mechanisms (along with other factors such as electrostatics) that have been proposed to govern protein sorting into different membrane domains (McIntosh and Simon, 2006; Idema et al., 2010).

As summarized by Phillips et al. (2009), two types of membrane deformations are commonly involved in membrane-mediated interactions: thickness deformation (e.g., hydrophobic mismatch) and midplane bending (Fig. 2). Although the magnitude of deformation energy can be comparable (e.g., ~10 k_BT), the length scales for the two types of deformation can be dramatically different. Thickness deformation is short-ranged and likely involves only two to three layers of lipids that surround the protein (Yoo and Cui, 2013), whereas midplane bending decays much slower and can span a broad range of 5–500 nm, depending on membrane tension (Phillips et al., 2009). In other words, midplane bending may lead to long-range interactions between membrane proteins. When two proteins are of the same

Departments of Chemistry, Physics and Biomedical Engineering, Boston University, Boston, MA.

Correspondence to Qiang Cui: qiangcui@bu.edu.

https://doi.org/10.1085/jgp.201812077

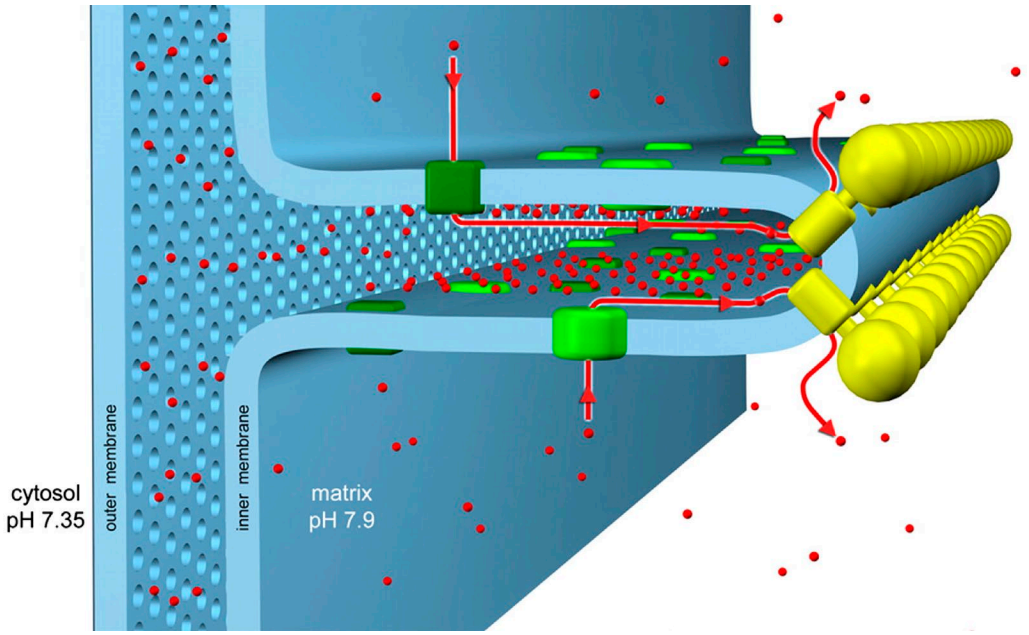

Figure 1. **Membrane-mediated protein–protein interactions.** Schematic illustration of protein organization in the mitochondrial cristae membranes (adapted from Fig. 5 of Davies et al., 2011); the ATP synthase dimers (yellow) assemble into rows along the cristae ridge, whereas proton pumps (green) reside predominantly in the at membrane regions. Such organization has been proposed to support a high local proton gradient (however, see the discussion by Rieger et al., 2014) and efficient ATP synthesis (Davies et al., 2011).

shape and thus induce midplane bending in the same direction, the elasticity-mediated interaction between them is intuitively attractive, because association leads to an overall reduction in the area of bent membrane. Previous theoretical analyses (Kim et al., 1998; Weikl et al., 1998) using linear elasticity models found, however, that two proteins within an isotropically bent membrane experience repulsive interactions, and attraction requires anisotropic curvature imprints and/or multibody interactions (Chou et al., 2001). The general validity of the prediction, however, is not guaranteed because the linearized continuum mechanics model may not be quantitatively reliable in the limit of large curvature (Reynwar et al., 2007; however, also see Yoo et al., 2013).

In the specific case of mitochondrial ATP synthase, it has been recognized that it forms a V-shaped dimer because of an inherent feature of the mitochondria-specific subunits (Davies et al., 2011); bacterial and chloroplast ATP synthases, in contrast, are monomeric. The unique V-shape of the dimer leads to significant midplane bending of the surrounding membrane (Fig. 2), as shown most directly in coarse-grained molecular dynamics simulations (Davies et al., 2012). Therefore, one would expect significant interaction between two (and multiple) dimers, although, as discussed above (Kim et al., 1998; Weikl et al., 1998; Chou et al., 2001; Reynwar et al., 2007), neither the sign (repulsion vs. attraction) nor the magnitude of the interaction is straightforward to predict. This challenge was explicitly addressed by Anselmi et al. (2018) using free energy simulations. Adopting a popular coarse-grained model (Marrink and Tieleman, 2013), which they tested explicitly for membrane elasticity, Anselmi et al. (2018) evaluated the free energy profile for the association of two ATP synthase dimers. Both the magnitude and range of the computed interaction are remarkable: the two dimers start to sense each other at a distance separation as far as 40 nm, and the binding free energy is ~18 kcal/mol (~30 k_BT). The free energy profile is essentially barrierless, which suggests that the association of the dimer is expected to be spontaneous. Evidently, the membrane-mediated interaction is sufficient to drive the assembly of ATP synthase dimers and leads to practically zero probability of observing

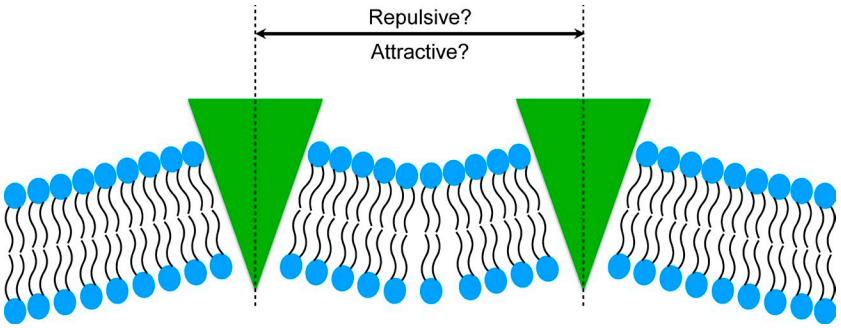

Figure 2. **Cone-shaped transmembrane proteins induce midplane membrane bending.** Midplane membrane bending leads to long-range (5–500 nm) interactions (Phillips et al., 2009) between proteins. Note that although linear elasticity theory predicts repulsive interactions between proteins of similar isotropic shape (Kim et al., 1998; Weikl et al., 1998; Chou et al., 2001), attractive interactions may arise because of curvature anisotropy and deviation from linear elasticity (Chou et al., 2001; Reynwar et al., 2007).

an isolated dimer, in qualitative agreement with experimental observations (Davies et al., 2011, 2012).

One interesting question concerns whether direct protein–protein interactions play a role in dimer assembly. In other cases, specific protein–protein interactions are known to be essential for the formation of ordered protein domains in the membrane (Jagannathan et al., 2002). In the specific case of ATP synthase, as discussed in more detail in the work of Anselmi et al. (2018), several protein–protein interactions proposed in older studies appear to be disputed by more recent structural evidence (Hahn et al., 2016). In this regard, the use of a coarse-grained model that features a rather rigid protein structure and simplified interactions among amino acids is, in fact, advantageous. The simulation clearly highlights that the association of ATP synthase dimers does not rely on highly specific or detailed protein–protein interactions, and that a membrane elasticity–mediated interaction alone is sufficient to drive the association.

Although assembly of ATP synthase dimers into rows reduces membrane bending along the direction of association, significant curvature remains in the perpendicular direction. It is thus conceivable that, as the inner membrane continues to grow because of lipid synthesis, the ridges aligned by the dimer rows prime membrane buckling and invagination, leading ultimately to the intricate structure of cristae. An additional role of the ATP synthase ridge, as proposed by Anselmi et al. (2018), is to prevent unregulated fusion or division of the inner mitochondria membrane at highly curved locations. Therefore, ATP synthase assembly and cristae formation appear to be highly coupled processes, although the biological function of ATP synthase is not membrane bending per se. In this regard, the ATP synthase assembly process is distinct from other protein systems whose function is to drive membrane remodeling, such as BAR domains, clathrin, and ESCRT complexes (Doherty and McMahon, 2008; Schöneberg et al., 2017). The assemblies of these systems are largely driven by specific protein–protein interactions, although membrane elasticity also contributes in certain cases (Simunovic et al., 2017).

The elegant analyses and model presented by Anselmi et al. (2018) describe the fundamental process of ATP synthase dimer assembly, using 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-choline (POPC) as the model membrane. In practice, other factors and mechanisms might further contribute to the robustness and regulation of the process. For example, specific lipids with large spontaneous curvatures such as phosphatidylethanolamine and cardiolipin (each may account for 25% of the inner membrane lipids) may further stabilize the membrane ridge formed by the row of dimers. Moreover, the protein/lipid ratio for the inner membrane is 80:20—much higher than the outer membrane—and thus the material properties of the membrane surrounding ATP synthases could be more complex, and other factors that involve protein/membrane interfaces may also contribute (McIntosh and Simon, 2006; Katira et al., 2016). Nevertheless, the recurring theme is that membrane protein assembly can be largely dictated by the statistical mechanics of the membrane and the protein/membrane interface, rather than direct protein–protein interactions (Reynwar et al., 2007). In this context, we echo the sentiment from Simunovic et al. (2017) that although theoretical models based on simplified continuum theories are highly valuable, their quantitative transferability to protein systems, in terms of predicting the sign, magnitude, and range of membrane-mediated protein–protein interactions, is not well known. Systematic analyses that integrate multiscale simulations and quantitative in vitro experiments (as also proposed by Anselmi et al., 2018) would be tremendously informative.

Acknowledgments

This work is supported in part by the National Science Foundation (grant NSF-DMS-1661900).

The author declares no competing financial interests.

Lesley C. Anson served as editor.

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