Cardiolipin puts the seal on ATP synthase

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ATP synthases are remarkable proteins that regenerate the molecular fuel for cellular processes in all domains of life (1). Embedded into the inner membranes of mitochondria, chloroplasts, and bacteria, F0F1-ATP synthase produces most of the cellular ATP, using ADP and inorganic phosphate as inputs (Fig. 1A). This thermodynamically unfavorable reaction is powered by a proton electrochemical gradient across the membrane, which drives the rotation of the c-ring in the F0 part of ATP synthase (2). The asymmetrical central shaft connecting F0 and F1 then deforms the three active sites in the F1 part to catalyze ATP synthesis (1). The proper function of this machine requires efficient rotation of the c-ring in a highly viscous lipid membrane without any proton leaks. This problem is compounded by the unusual structure of the a-subunit facing the c-ring, with its long helices parallel to the membrane distorting the lipid packing around the F0 part (3, 4).

In PNAS, Duncan et al. (5) report that an unusual lipid–protein interaction in the F0 part of ATP synthase is central to efficient c-ring rotation with properly sealed c-ring/a-subunit/membrane interfaces (Fig. 1A). Until quite recently, membrane proteins were assumed to float rather freely within biological membranes. This view has been challenged by rapidly mounting evidence for specific interactions between lipids and membrane proteins from a variety of methods, including light spectroscopy, X-ray crystallography, electron microscopy, and molecular dynamics (MD) simulations (6). Lipids are now recognized as major factors in membrane protein assembly, supercomplex stabilization, function, and regulation.

The inner mitochondrial membrane is no exception. In fact, this membrane contains an unusual anionic lipid, cardiolipin (CL), formed by two phosphatidyl groups linked by a glycerol moiety (7) (Fig. 1B). As the fingerprint phospholipid of mitochondria, it plays a critical role in regulating oxidative phosphorylation (OxPhos). CL is now recognized as a prerequisite for optimal activity of respiratory chain proteins, including complex I (NADH/quinone oxidoreductase), complex III (cytochrome bc1), complex IV (cytochrome c oxidase), and complex V (ATP synthase). A number of possible functions have been proposed for CL in OxPhos, ranging from protein rigidification to proton transfer (8). CL–protein interactions in the inner mitochondrial membrane are not limited to the OxPhos complexes, as witnessed by a rapidly growing list of other proteins interacting specifically with CL, ranging from ADP/ATP carriers to translocases (9, 10). In reflection of the important role of these interactions for the function of mitochondria, deficiency of CL has been associated with several diseases, particularly Barth syndrome (11).

Interaction between ATP synthase and CL is an old story given a new life. Already in 1973, Santiago et al. (12) established a correlation between the presence of CL and the level of ATP synthase activity in mitochondria. Two decades later, solid-state NMR (ssNMR) suggested a high affinity interaction between F0F1-ATPase and CL (13). Having stayed relatively quiet for another two decades, the field was reactivated in 2011, when mass spectroscopy revealed CL bound tightly to the K-ring membrane rotor of V-type ATPases. Binding was suggested to be on the inside of the ring, with 1:1 stoichiometry of K-ring subunits and CL (14). In the same year, electron tomography indicated a pivotal role for CL in the oligomerization of ATP synthase, which, in turn, affects cristae morphology in mitochondria (15). Subsequently, ssNMR showed that CL binds to the c-rings of ATP synthase from Escherichia coli and Streptococcus pneumonia (16), but binding was suggested to occur on their outside surfaces this time. First, the conserved lysine as the potential binding site of CL is located on the outside surface, and, second, the c6-ring of the mammalian ATPase has insufficient space within its annulus.

Taking advantage of recent advances in coarse-grained (CG) MD simulation, Duncan et al. (5) now give us a detailed view of the structure and dynamics of c-ring/CL interactions. In MD simulations of the bovine c6-ring in CL-containing membranes, and of bacterial c10- and c11-rings, they found a remarkable interaction pattern. CL consistently binds to the c-rings considerably longer than phosphatidyl lipids, especially in the inner leaflet of the membrane. Around

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The bovine c₆-ring, CL tends to cluster selectively near a group of residues in the inner leaflet centered at a conserved, fully trimethylated lysine (K43, Q44, Q45, and S48), and to a lesser extent in the outer leaflet around K7. The phosphatidyl lipids, by contrast, are less organized around the c-rings. Somewhat surprisingly, the CL binding to the c-rings is not nearly as tight as to other mitochondrial complexes studied with similar methods. Arnarez et al. (17, 18) showed earlier that CL binds tightly into the highly specific binding sites in complex III and complex IV with long residence times, staying continuously bound for the entire simulation (>50 μs) in some cases. By contrast, during the c-ring simulations (5), CL binds and unbinds several times on a microsecond time scale.

The results of Duncan et al. (5) have major implications for our understanding of Fₒ action. Efficient c-ring rotation demands minimal friction with the surrounding membrane. Tightly bound CL, with the long residence times reported for cytochrome c oxidase and cytochrome bc₁ (17, 18), could be unfavorable. Such tight interactions should interfere particularly with the functionally required rotation of the c-ring past the α-subunit. A tightly bound lipid would lock the rotor in a manner similar to some inhibitors (19). However, selective binding of CL to the c-ring appears to be required for the function and assembly of ATP synthase. The results of Duncan et al. (5) help resolve this paradox: CL binds selectively but, at the same time, intermittently. In complexes III and IV, CL appears to act as a bridging glue; by contrast, it acts here as a lubricant.

The glycerol bridge provides the required flexibility for CL to interact with these very different surface shapes. CL can sit in a concave groove of cytochrome c oxidase or transiently bind onto the convex surface of the c-ring. Stabilized further by interactions of its anionic headgroup with positively charged residues, the fatty acyls sit on the smooth surface of the c-ring, thereby reducing friction.

Looking forward, we can expect further exciting developments in the CL story. In CG-MD simulations using the MARTINI force field (5, 17, 18), groups of usually four heavy atoms are represented by a single interaction center. Such coarse graining describes lipid–protein interaction at a reasonable level of detail and makes it possible to simulate biomolecular systems on time scales normally inaccessible to fully atomistic simulations. Nevertheless, details of specific interactions are lost, particularly between charged groups. Furthermore, the localization of lipids around membrane

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**Fig. 1.** Schematic of Fₒ,F₁-ATP synthase function. (A, Top) Proton translocation mediated by the c-ring of the membrane-embedded Fₒ part drives rotation. (A, Bottom) The asymmetrically shaped rotor axis attached to the c-ring distorts the active sites in the F₁ part, where ATP is synthesized from ADP and inorganic phosphate Pᵢ. Simulations by Duncan et al. (5) show that CL, an abundant lipid in the inner mitochondrial membrane, accumulates near the c-ring. The structure is based on Protein Data Bank ID code 5FIK (4). (B) CL is an unusual lipid with four acyl chains.
proteins is affected by the flexibility of the protein surface itself (20), which may not be captured fully in the CG-MD simulations. Although atomistic simulations are currently limited to runs of a few microseconds, powerful supercomputers combined with new algorithms will soon allow us to probe lipid–protein interactions at atomic resolution over relevant times. From such atomistic MD simulations, we can expect additional insight into the specificity of the interactions and quantitative residence time estimates, as well as a full accounting of the protein flexibility. Last but not least, by using the already available medium-resolution structures of complete ATP synthases, and the eagerly awaited high resolution structures, simulations should shed new light on the functional roles of CL (21) and allow us to examine a proposed role of CL in ATP synthase oligomerization and the formation of mitochondrial cristae (15). Duncan et al. (5) point the way for both experimentation and computation to explore one of the most fundamental questions of molecular membrane biology: protein–lipid interactions in ATP synthase and beyond.

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