mutations exhibited somewhat diverse mobility in SDS-PAGE but were all below the wild-type monomer band. All of the AxAxAxA mutants exhibited reduced stability of the *c*-ring; the A18G mutant was least affected and the quadruple mutant was among the most affected. These observations are discussed in the context of catalytic properties and emerging structural information about the alkaliphile c-ring.

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2P.27 Cyclophilin D interaction with the ATP synthase oligomeric forms

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The mitochondrial F_OF_1ATP synthase is a rotary enzyme organized into a catalytic (F_1) and a membranous moiety (F_O) linked by central and peripheral stalks. The core mammalian enzyme consists of 16 conserved subunits, the F_1 subunits α–ε, the inhibitor protein IF_1 and the F_O subunits a–g, OSCP, A6L and F_6. The complexity of such structural organization requires accessory factors, whose definition is still under investigation. We recently demonstrated that in mitochondria cyclophilin (CyP) D, a well characterized mitochondrial chaperone, is associated to ATP synthase interacting with the enzyme lateral stalk, which is formed by the F_O subunits b, d, F_6 and OSCP. CyPD function does not appear to be related to enzyme assembly, which is not affected in CyPD-null mitochondria. Conversely, the ATP synthase–CyPD interactions have functional consequences, so that increased CyPD binding decreases enzyme catalysis and *vice versa* [1]. Involvement of the lateral stalk raises the question to which form of ATP synthase CyPD is bound. In fact, although this complex is commonly isolated as a functional monomer, different lines of evidence support that dimers/oligomers of ATP synthase exist in membrane and are stabilized by subunits of both F_O, i.e. subunits a, b, c, e, g, and F_1, i.e. IF_1 [2]. Treatment of whole mitochondria or submitochondrial particles from bovine heart with 2–10 mM oligomycin, which binds to the F_O subunit a, affected the supra-molecular organization of ATP synthase and the percentage of dimers/oligomers purified by digitonin and separated by Blue-native PAGE were inversely related to oligomycin concentration. Immunoprecipitation showed a parallel reduction of the amount of CyPD associated to ATP synthase. Accordingly, treatment of the membranes with *n*-dodecyl-maltoside, which destabilized the oligomeric forms during extraction and generated only monomers in Blue-native PAGE, significantly reduced CyPD bound to ATP synthase with respect to the treatment with digitonin in the absence of oligomycin. These data demonstrate that the dimer/oligomer formation strongly stabilizes CyPD binding to the lateral stalk, supporting a functional role of CyPD as modulator of the dimers/oligomers, which would represent the physiological form of ATP synthase [2].

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2P.28 Structural studies of the membrane-embedded c-ring of the F_1F_O-ATP synthase from a thermoalkaliphilic bacterium reveal a strategy for adaptation to alkaline environments

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F_1F_O-ATP synthases are bipartite molecular motors able to convert an electrochemical membrane potential into ATP by a rotational mechanism. F_1 ($\alpha_3\beta_3\gamma\delta\varepsilon$) contains the catalytic subunits while F_O (ab_2c_{10-15}) includes those embedded in the membrane. Within the rotor ($c_{10-15}\gamma\varepsilon$), the c-subunits are assembled in a ring of variable size in different species, comprising from 10 to 15 subunits. The c-ring is crucial as it transduces the energy gained from ion translocation across the membrane into conformational changes in the $\alpha_3\beta_3$-headpiece, thus catalyzing ATP synthesis. The stoichiometry of the ring determines the number of translocated ions required per ATP synthesized and therefore is an important parameter of the cell bioenergetics. Here, we have used *E. coli* to heterologously express the ATP synthase from the thermoalkaliphilic *Caldalkalibacillus thermarum* (formerly *Bacillus* sp.) strain TA2.A1, and show that it is fully assembled and functional. From this high-yield expression, we have isolated the c-ring and determined its stoichiometry to be 13, using various techniques. These include a novel mass-spectrometry method, termed "laser-induced liquid bead ion desorption" (LILBID), which allows the mass determination of non-covalently assembled, detergent-solubilized membrane protein complexes. In addition, AFM imaging, cryo-EM of 2D crystals and X-ray diffraction of 3D crystals also demonstrate that this c-ring harbors 13 c-subunits. Analysis of the c-ring isolated from wild-type cells is consistent with this result, demonstrating that the stoichiometry of a c-ring is a property of its primary sequence. Our structural analysis also reveals a special feature of this c-ring, namely an unusually distant packing of the inner helices. A conserved GxGxGxGxG motif, known to permit close packing of transmembrane helices, is altered to AxGxSxGxS in this strain, and appears to change the inter-c-subunit contacts, thereby enlarging the ring diameter to host a greater number of c-subunits. Comparative sequence analysis shows that this altered glycine-motif is also a feature of c-subunit sequences in many other alkaliphilic members of the *Bacillaceae* family. We propose that the enlargement of the c-rings in H^+-dependent F-ATP synthases is an adaptation to facilitate ATP synthesis at low proton-motive-force, which typically occurs in bacteria growing at alkaline pH. Further structural studies of the holo-enzyme by X-ray crystallography and single-particle EM are underway.

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