Structure and mechanism of the ATP synthase membrane motor inferred from quantitative integrative modeling.

Two subunits within the transmembrane domain of the ATP synthase—the *c*-ring and subunit *a*—energize the production of 90% of cellular ATP by transducing an electrochemical gradient of H^+ or Na^+ into rotational motion. The nature of this turbine-like energy conversion mechanism has been elusive for decades, owing to the lack of definitive structural information on subunit *a* or its *c*-ring interface. In a recent breakthrough, several structures of this complex were resolved by cryo–electron microscopy (cryo-EM), but the modest resolution of the data has led to divergent interpretations. Moreover, the unexpected architecture of the complex has cast doubts on a wealth of earlier biochemical analyses conducted to probe this structure. Here, we use quantitative molecular-modeling methods to derive a structure of the *a*–*c* complex that is not only objectively consistent with the cryo-EM data, but also with correlated mutation analyses of both subunits and with prior cross-linking and cysteine accessibility measurements. This systematic, integrative approach reveals unambiguously the topology of subunit *a* and its relationship with the *c*-ring. Mapping of known Cd^{2+} block sites and conserved protonatable residues onto the structure delineates two noncontiguous pathways across the complex, connecting two adjacent proton-binding sites in the *c*-ring to the space on either side of the membrane. The location of these binding sites and of a strictly conserved arginine on subunit *a*, which serves to prevent protons from hopping between them, explains the directionality of the rotary mechanism and its strict coupling to the proton-motive force. Additionally, mapping of mutations conferring resistance to oligomycin unexpectedly reveals that this prototypical inhibitor may bind to two distinct sites at the *a*–*c* interface, explaining its ability to block the mechanism of the enzyme irrespective of the direction of rotation of the *c*-ring. In summary, this study is a stepping stone toward establishing the mechanism of the ATP synthase at the atomic level.