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.