MS8-O2 The structure and function of calcium activated lipid scramblases and ion channels of the TMEM16 family

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The TMEM16/Anoctamin family of membrane proteins is broadly expressed in eukaryotes and features a remarkable functional diversity. The family contains the long sought-after Ca^{2+}-activated chloride channels but also lipid scramblases or cation channels. We have determined the crystal structure of nhTMEM16, a fungal family member that operates as a Ca^{2+}-activated lipid scramblase [1]. Each subunit of the homodimeric protein encompasses ten transmembrane helices and structured cytosolic domains at the N- and C-terminus. The 'subunit cavity', a hydrophilic membrane-traversing cavity contained within each subunit that is exposed to the lipid bilayer acts as potential site of catalysis in both functional branches of the family. This cavity is sufficiently wide to accommodate the polar head groups of lipids and it contains residues that, upon mutation in the Cl^--channel TMEM16A, affect its ion selectivity. The 'subunit cavity' harbors a Ca^{2+}-binding site located within the hydrophobic core of the membrane. In this site two Ca^{2+} ions are coordinated by six conserved residues, five of which carry a negative charge. Mutations of these residues decrease the potency of Ca^{2+} in the activation of lipid scrambling in nhTMEM16 and ion conduction in TMEM16A. The nhTMEM16 structure thus reveals the general architecture of the family and its mode of Ca^{2+}-activation. It also provides insight into potential scrambling mechanisms and serves as a framework to unravel the conduction of ions in certain TMEM16 proteins acting as ion channels.

[1] Brunner, J.D., et al., *X-ray structure of a calcium-activated TMEM16 lipid scramblase.* Nature, 2014. **516**(7530): p. 207-12.

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MS8-O3 Crystal Structure of Mitochondrial Respiratory Complex I

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Proton-pumping complex I is the largest membrane protein complex of the mitochondrial respiratory chain. The enzyme substantially contributes to energy conversion in eukaryotic cells as it couples oxidation of NADH and reduction of ubiquinone to pumping of four protons. It thus contributes to the generation of the electrochemical proton gradient across the inner mitochondrial membrane, which drives ATP synthesis. Complex I is a major source of deleterious reactive oxygen species (ROS) and its dysfunction is associated with many inborn and degenerative disorders.

We determined the X-ray structure of mitochondrial complex I (41 subunits and about 1MDa) from the strictly aerobic yeast *Yarrowia lipolytica* and shed light on its mechanism (1,2). With brominated competitive inhibitors and anomalous diffraction, we identified the binding site of the hydrophobic substrate quinone deeply buried in the hydrophilic domain of the complex. The site is connected to four putative proton translocation paths via a continuous axis of acidic and basic residues that runs centrally through the entire membrane embedded arm. The structure provides clues that the spatially separated redox reactions and proton pumping machinery are linked via conformational changes at and close to the quinone binding pocket. The same structural rearrangements may explain the active/deactive transition of complex I. The reversible transition is discussed as protection mechanism against excessive ROS formation in many eukaryotic species. Slow return to the A form was shown to attenuate reperfusion injury.

The function of many of the 30 accessory subunits of the mitochondrial complex I is not known. We identified the position of a single zinc ion present in *Y. lipolytica* complex I by anomalous diffraction and thereby located NUMM. This accessory subunit was shown to be required for the assembly of iron-sulfur cluster N4, a very late assembly stage of the complex (3).

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