The cytochrome c complexes, like the cytochrome bc₁ (complex III) and the cytochrome cbb₃ (a bacterial type complex IV), are essential for major energy transduction pathways such as respiration and photosynthesis. These oligomeric membrane-integral complexes contain c-type cytochrome subunits as well as other subunits carrying different cofactors, like the iron–sulfur clusters and heme–copper (heme b₃–Cus₃) centers. Their biogenesis pursues multi-stepped processes involving independent maturation of cofactor containing subunits, and subsequent assembly of mature subunits to produce active complexes. These steps are precisely coordinated both temporally and spatially to yield functional complexes and to avoid accumulation of toxic intermediates. The cbb₃-type oxygen reductase (cbb₃-type cytochrome c oxidase) with its CcoN subunit that carries a heme b₃–Cus₃ cytochrome and its CooO and CcoP subunits that are monoheme and diheme c-type cytochromes, respectively, provides an excellent paradigm for investigating the biogenesis of cytochrome c complexes. We will discuss the cytochrome c maturation process (Ccm-system I) that produces the c-type cytochrome subunits of the cytochrome cbb₃, as found in Rhodobacter capsulatus. The Ccm-system I is formed of nine (CcmABCDDEFGHI) membrane proteins that work together to achieve the stereo-specific thioether bond formation between the vinyl groups of heme b (protoporphyrin IX–Fe) and the thiol groups of apocytochromes c heme-binding site (C₁XXC₂H) cysteine residues. We will then present our current knowledge on the formation of the heme b₃–Cus₃ center of the CcoN subunit of cytochrome cbb₃. In particular, we will describe the emerging pathway of Cu import into the cytoplasm and its delivery to CcoN. Finally, we will address the issue of how the subunits of cytochrome cbb₃ are assembled, and further, how the individual cytochrome c complexes come together to form larger macromolecular entities. Both naturally occurring and genetically modified functional supercomplexes capable of sustaining efficient photosynthesis and respiration will be described.

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P3 & P4 — ATP synthase and respiratory complexes

P3 & P4.1

New features of ATP synthases

John E. Walker

MRC Mitochondrial Biology Unit, Wellcome Trust/MRC Building, Hills Road, Cambridge CB2 0XY, UK
E-mail: walker@mrc-mbu.cam.ac.uk

The F-ATP synthases in eubacteria, chloroplasts and mitochondria have many common features; they consist of two rotary motors linked by a stator and a flexible rotor. When the rotor is driven by proton motive force, the direction of rotation ensures that ATP is made from ADP and phosphate in the globular catalytic domain. When ATP is hydrolysed in the catalytic domain, the rotor turns in the opposite sense and protons are pumped through the membrane domain, away from the catalytic domain. However, some eubacterial enzymes cannot hydrolyse ATP. The ATP synthases differ in how they are regulated. Eubacteria have evolved a range of mechanisms, and the chloroplast enzyme is rendered inactive by a redox mechanism in the hours of darkness. Mitochondria contain an inhibitor protein, I1#, that inhibits ATP hydrolysis but not ATP synthesis. The inhibitory region is intrinsically unstructured, and the pathway of how it folds and binds to the enzyme has been defined by mutational and structural analysis. However, its in vivo role remains mysterious. The ATP synthases from mitochondria, eubacteria and chloroplasts differ fundamentally in the energy cost that they pay to make each ATP molecule. This is related directly to the number of c-subunits that form the hydrophobic ring in the enzyme’s rotor: the smaller the ring, the lower the energy cost of producing each ATP molecule. The most efficient ATP synthase is found in the mitochondria of multicellular animals, where eight c-proteins form the ring. A lysine residue in c-subunits from multicellular, but not unicellular species, is trimethylated. The significance of this modification will be discussed. In 2013, Bernardi and colleagues proposed that the mitochondrial ATPase is involved in the mitochondrial permeability transition. This transition is triggered by cofactors. Covalent addition of FAD in the Sdh1 subunit is facilitated by the (SDHAF2) assembly factor. Two novel assembly factors Sdh6 (SDHAF1) and Sdh7 (SDHAF3) facilitate the maturation of Sdh2, which includes the insertion of three FeS clusters (2Fe–2S, 4Fe–4S and 3Fe–4S centers). Yeast lacking either Sdh6 or Sdh7 are partially impaired in the maturation of Sdh2, but a marked impairment is seen in a double mutant strain. The dependency of SDH assembly on the Sdh6 and Sdh7 factors is especially marked in respiratory cultures. The mutant cells exhibit a marked instability in the FeS subunit Sdh2. In cells lacking the membrane anchor subunits, Sdh6 and Sdh7 are associated with the two catalytic subunits Sdh1 and Sdh2 in an assembly intermediate. Genetic suppressors of the respiratory defect of cells lacking either Sdh6 or Sdh7 were recovered and identified as Yap1, which activates the expression of anti-oxidant genes. The SDH defect seen in sod6 or sod7 mutant cells is reversed by exogenous antioxidants. We propose that Sdh6 and Sdh7 function as Sdh2 chaperones stabilizing the subunit during maturation. Sdh6 and Sdh7 appear to protect the FeS-Sdh2 against endogenous oxidants prior to its association with the membrane anchor subunits associate to form the holo-enzyme. Drosophila lacking the Sdh7 ortholog SDHAF3 is hypersensitive to oxidative stress and exhibits muscular and neuronal dysfunction. The human Sdh6 ortholog SDHAF1 has a conserved function in chaperoning the FeS subunit during SDH biogenesis.

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P2.3

Assembly of succinate dehydrogenase: Interplay between iron–sulfur cofactor insertion and subunit assembly

Un Na¹, Wendou Yu¹, James Cox¹, Knut Brockmann¹, Carl S. Thummel¹, Dennis R. Winge²

¹University of Utah Health Sciences Center, Salt Lake City, UT 84132, USA
²University Medical Center, Georg August University, Göttingen, Germany
E-mail: dennis.winge@hsc.utah.edu

Succinate dehydrogenase (SDH) is an integral component of the mitochondrial respiratory chain that catalyzes the oxidation of succinate to fumarate. The tetrameric enzyme contains 5 redox cofactors including a covalently bound FAD and 3 FeS clusters in a hydrophilic segment consisting of Sdh1 and Sdh2 subunits and a membrane anchor domain consisting of the other two subunits. The biogenesis of SDH is dependent on assembly factors for the insertion of redox
opening a large membrane pore, potentiated by inducers such as the phosphate, the depletion of adenine nucleotides and the presence of thiol oxidants. It leads to the swelling of the mitochondria, loss of proton motive force, disruption of ion homeostasis and hydrolysis of ATP by the ATP synthase. These events have been linked to pathways leading to cell death, and to human diseases including cardiac ischemia and muscle dystrophy. We have examined this proposal, and a summary of our findings will be presented.

References

P3 & P4.3

Electron cryomicroscopy investigation of the proton-conducting pore of ATP synthase
John Rubinstein
The Hospital for Sick Children Research Institute, 686 Bay Street, Rm. 20-9705, Toronto, ON, Canada
E-mail: john.rubinstein@utoronto.ca

Adenosine triphosphate (ATP) synthases use the energy stored in a transmembrane proton-motive force to synthesize ATP from adenosine diphosphate (ADP) and inorganic phosphate. The translocation of protons across the membrane region of the complex leads to rotation of a rotor subcomplex that drives conformational changes in the enzyme’s membrane-extrinsic catalytic region. The mechanism by which proton translocation induces rotation is not known. Here, we present structural data from single particle electron cryomicroscopy (cryo-EM) that gives insight into the architecture of the membrane-bound motors of ATP synthases and related enzymes. This evidence supports a two-half channel model for proton translocation.

References