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Abstracts

S1 Respiratory Chain and Photosystems

Lectures

1L.1 Electron transfer routes in cyanobacterial thylakoid membranes — Impact on biohydrogen production

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Optimization of electron transfer from water to biohydrogen production in a model organism *Synechocystis* sp. PCC 6083 involves several steps. PSII function can be improved by introducing a proper PSII reaction center D1 protein (encoded by the *psbA* gene family). To this end, an expression of a specific *psbA* gene that encodes a D1′ protein was detected under anaerobic conditions. We have also demonstrated a novel and crucial function for Flavodiiron (FDP) proteins Flv2 and Flv4 in photoprotection of PSII. The rate of accumulation of *flv2* and *flv4* transcripts upon shift of cells from high to low CO_2 is strongly dependent on light intensity. Characterization of FDP inactivation mutants revealed a specific decline in PSII centers and impaired translation of the D1 protein in Δflv2 and Δflv4 when grown at air level CO_2 whereas at high CO_2 the FPDs were dispensable. Δ*flv2* and Δ*flv4* were also more susceptible to high light induced inhibition of PSII than WT or Δ*flv1* and Δ*flv3*. Of the four flavodiiron proteins (Flv1–4) in Synechocystis 6803, a physiological function of Flv1 and Flv3 is in the Mehler reaction. Up to 30% of electrons derived from water by PSII may be directed to molecular oxygen *via* Flv1 and Flv3, and thus this route might seriously compete for electrons with the hydrogenase. Besides FDPs, the multiple NDH-1 complexes in cyanobacterial thylakoid membranes have a crucial role in electron transfer reactions, particularly in cyclic electron transfer around PSI, in respiratory electron transfer and in carbon concentrating mechanisms. Moreover, interplay between the FDPs and NDH-1 complexes is demonstrated to occur in electron transfer reactions.

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1L.2 Structural and functional insights into mitochondrial complex I

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The molecular mechanism how in complex I (NADH:ubiquinone oxidoreductase) proton translocation is linked to electron transfer is still unknown. Using the strictly aerobic yeast *Yarrowia lipolytica* as a powerful genetic system to study mitochondrial complex I, we have explored the ubiquinone reducing catalytic core of complex I by extensive site-directed mutagenesis. This functionally central region of complex I is located at the interface between the 49 kDa and the PSST subunit of the peripheral arm, where iron–sulfur cluster N2 serves as the immediate reductant of ubiquinone. We have located the likely entry pathway for ubiquinone leading to a conserved tyrosine located next to cluster N2 and investigated the role of the isoprenoid side chain for the interaction of ubiquinone with complex I. We have also mapped domains interacting with representatives of the different classes of hydrophobic complex I inhibitors. New evidence on the location of the ubiquinone binding pocket within complex I and the path leading to the site where the hydrophobic substrate is reduced will be discussed. We propose that long range conformational changes drive proton pumping of complex I through a two-state stabilization change mechanism involving distinct binding modes of charged ubiquinone intermediates.

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1L.3 High resolution structure of the electron input side of the respiratory complex I

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The respiratory complex I couples the electron transfer from NADH to ubiquinone with the translocation of protons across the membrane. The three-dimensional structure of the peripheral arm of *Thermus thermophilus* complex I was recently determined at a resolution of 3.3 Å, revealing the putative electron transfer pathway. The mode of interaction of nicotinamide nucleotides with the complex is important to understand because it was proposed that nucleotide binding results in conformational rearrangements essential for energy conservation by coupling redox processes to active proton pumping. In order to study the interaction and binding of nucleotides and inhibitors to the NADH binding site of complex I, we have heterologously overproduced and crystallized the electron input module of the enzyme from the hyperthermophilic bacterium *Aquifex aeolicus*, consisting of subunits NuoE and NuoF, in *E. coli*. The preparation contains the NADH binding site and the FMN as well as the binuclear Fe/S cluster N1a and the tetranuclear cluster N3. The structures of a native form, a form with bound nucleotides, and forms with several inhibitors were solved at resolutions up to 1.9 Å, revealing the molecular details of the binding and interaction modes of the coenzyme. Cluster N1a was proposed to be implicated in an intricate mechanism to minimize the generation of reactive oxygen species: After one electron is passed from the reduced FMN cofactor to the tetranuclear cluster N3 and further down the

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electron transport chain, the second electron is stored in N1a to shorten the lifetime of the FMN semiquinone radical that might react with abundant dioxygen to generate the hazardous superoxide anion. To prove this hypothesis, a variant of the module missing cluster N1a was produced.

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1L.4 Progress towards the molecular mechanism of mitochondrial complex I

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Complex I (NADH:ubiquinone oxidoreductase) is crucial to respiration in many aerobic organisms. In mitochondria it oxidises NADH (regenerating NAD^+ for the tricarboxylic acid cycle and fatty-acid oxidation), reduces ubiquinone (the electrons are then used to reduce oxygen to water), and transports protons across the mitochondrial inner membrane (contributing to the proton motive force that supports ATP synthesis and transport processes). Complex I is also a major contributor to cellular reactive oxygen species production. Our approach to determining the reaction mechanism of complex I is to consider it in several simpler parts that can be tackled and defined individually, before being recombined to produce the complete picture. Thus, the mechanism of complex I comprises four sequential steps. Two steps, NADH oxidation by the flavin mononucleotide, and intramolecular electron transfer from the flavin to bound quinone (along a chain of iron–sulphur clusters), are increasingly well understood. Conversely, the mechanisms of quinone reduction and proton translocation (including the possible involvement of semiquinone species in reactive oxygen species production) are very poorly understood. This talk will present and discuss recent data that address the mechanisms of quinone reduction and proton translocation by complex I.

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1L.5 Mitochondrial respiratory chain super-complex I–III in physiology and pathology

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Recent investigations by native gel electrophoresis showed the existence of supramolecular associations of the respiratory complexes, confirmed by electron microscopy analysis and single particle image processing. Flux control analysis in our laboratory demonstrated that Complex I and Complex III in mammalian mitochondria kinetically behave as a single unit with control coefficients approaching unity for each component, suggesting the existence of substrate channeling within the super-complex. On the other hand Complex II and Complex IV appear kinetically independent in mammalian mitochondria. Reconstitution studies demonstrate that the formation of the supramolecular unit comprising Complex I and Complex III (super-complex I–III) largely depends on the lipid content and composition of the inner mitochondrial membrane: at high lipid content or with peroxidized lipids the super-complex association is impaired, as demonstrated by electrophoretic and kinetic analysis. The function of the super-complexes appears not to be restricted to kinetic advantages in electron transfer: we discuss evidence on their role in the stability and assembly of the individual complexes, particularly Complex I, and in preventing excess oxygen radical formation or anyway in changing the sites of superoxide generation. There is increasing evidence that disruption of the super-complex organization leads to functional derangements responsible for pathological changes, as we have found in K-ras-transformed fibroblasts, where loss of the highest molecular weight super-complexes is associated with enhanced formation of reactive oxygen species and strongly diminished Complex I activity.

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1L.6 A stochastic approach of the electron transport in the mitochondrial respiratory chain

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A stochastic approach is particularly well adapted to describe the time course of the redox reactions that occur inside the respiratory chain complexes because electron(s) inside a given complex is (are) alone. Accordingly we approach, using the Gillespie method, the molecular functioning of the bc_1 complex based on its known crystallographic structure and the midpoint potential of redox centres. The main features of our simulations are the dominant and robust emergence of a Q-cycle mechanism and the near absence of short-circuits in the normal functioning of the bc_1 complex. The bifurcation of the QH_2 electrons in Qo is due to the fact that the passage of the 'second' electron on b_L traps the 'first' on the FeS centre. However, this simple model fails to explain the antimycin inhibition of the bc_1 complex and the accompanying increase in ROS production. To obtain inhibition, we show that it is necessary to block the return of the electron from the reduced haem b_L to Qo. With this hypothesis a sigmoid inhibition by antimycin is observed. We also use this approach to describe the molecular functioning of the hydrophilic domain of complex I. We show that most of electrons take the route defined by $NADH^+$ site – FMN – N3 – N1b – N4 – N5 – N6a – N6b – N2 – Q site but frequently jump back and forth between neighbouring redox centres with the result that the net flux of electrons through complex I is far smaller than the number of redox reactions which actually occur. We also hypothesize that the additional N1a redox centre could have a role in reducing the life time of the flavine semiquinone thus limiting the ROS production.

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