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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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