Oxidative Phosphorylation & Mitochondrial Metabolism

3812-Pos

Coupled Electron and Proton Transfer in Complex I and Complex IV of the Respiratory Chain: Insights from Computer Simulations

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I will discuss coupled transport of electrons and protons in two key enzymes of the electron transport chain of aerobic cells: NADH dehydrogenase (Complex I) and cytochrome c oxidase (Complex IV), which are, respectively, the entry point, and the terminal enzyme in the respiratory chain.Computer simulations and theoretical modeling of ET/PT reactions in these enzymes provide important insights into the molecular mechanisms of these redox-driven proton pumps.

3813-Pos

Electrophysiology of Functional Coupling of Electron Transport Chain Complexes

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Electron transport chain in the inner mitochondrial membranes consists of four multisubunit protein complexes CI to CIV which are coupled via electron carrier molecules as well as protein-protein interactions. The coupling of the complexes is essential for the proper functioning of the chain and may be an important factor for regulation and balancing of respiration, ATP synthesis and production of reactive oxygen species. However, direct functional studies on the action of the respiratory chain in native surroundings are limited due to the poor accessibility via standard electrophysiological equipment. We performed electrophysiological analyses of electron transport chain in native inner mitochondrial membranes using the solid-supported membranes (SSM) and the SURFE^{2}R technology. The inner mitochondrial membranes were purified from pig heart mitochondria by sucrose gradient fractionation and adsorbed onto SSM sensors. The chain complexes were activated either by NADH for the studies of CI-CIII-CIV coupling, or by succinate and cytochrome *c* for the analysis of the CII-CIII function. The tested proteins were pharmacologically characterized using specific substrates and inhibitors. Serial application of different inhibitors as well as the coenzyme Q analogs decylubiquinone revealed a tight functional interplay between the complexes CI, CIII, and functional coupling to the complex CIV. The complexes CII and CIII were also functionally coupled. An excess of the coenzyme Q analog idebenone had stimulating effect on the CII-CIII activity but was reducing the CI-CIII-CIV-specific currents. In summary, the presented results demonstrate an easy and reliable approach for studying the complex functional interplay of mitochondrial transport proteins in their native environment, and can help to understand the physiology of different mitochondrial functions. Since different assay conditions can be tested on the same sensor, the technology allows highly effective comparative analysis of the different complex activities.

3814-Pos

Flash Initiated Redox Events within Cytochrome bc1 Suggest Equilibration between Hemes b: Effects of Temperature, Viscosity, Inhibitors and Substrates

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As an integral member of all major electron transfer chains, cytochrome bc_1 functions as a proton-motive pump; transferring protons across the membrane via oxidation of quinol substrates. Though a Q-cycle establishes the general consensus mechanism for the enzyme, several key questions remain. Namely, the nature of electron bifurcation at the Q_o site, and the potential for intermonomer electron equilibration and communication across the dimer. Through rapid, flash initiated oxidation of a cytochrome c surrogate, Ru_2D, we have investigated electron transfer between the iron sulfur center and cytochrome c_1; as well as the subsequent redox events of the cytochromes b via turnover at the Q_o site. Moreover, by modulating the extent of b heme reduction prior to flash initialized events, we are able to study the re-oxidation of heme b_H over an extended range of conditions. Herein, we report the effects of such variables as temperature, viscosity, inhibitors and substrates on the flash initiated redox events of cytochrome bc_1, with an emphasis on the redox events of the b hemes. Toward such ends, we show that a single turnover at the Q_o site can effectuate the oxidation of two equivalents of heme b_H, suggesting equilibration between the two low potential chains. This work was supported by NIH Grant GM20488 and RR15569.

3815-Pos

In Yarrowia Lipolityca Mitochondria the Association of NADH Dehydrogenase Type II with the Cytochrome Complexes Depends on the Growth Phase

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In Yarrowia lipolytica mitochondria, the electron transport from NADH to O2 is branched by alternative respiratory components. One external NADH dehydrogenase (NDH2e) and an alternative oxidase (AOX). Both enzymes are peripheral single-subunit oxido-reductases not implicated in proton gradient formation. Thus, if electrons pass through those two enzymes, the oxidation of NADH is not able to conserve energy. During exponential growth, this is undesirable; however, during the stationary phase this process may help to maintain a high rate of oxygen consumption. To prevent the electron flux between alternative components, either AOX may be interacting with the complex I or NDH2e with complexes III and IV. We have evaluated the participation of the alternative components on electron transport and on supramolecular structures of mitochondria from wild type and a αnubm mutant, where complex I is inactive and NDH2 was redirected to the matrix side (NDH2i)4. In order to determine whether there are specific interactions between NDH2e and other respiratory complexes, we measured oxygen consumption rates with different respiratory substrates and inhibitors. We suggested an interaction of NDH2e (but not NDH2i) with cytochrome complexes, indicating that the interaction sites are located in the intermembrane face of the cytochromic complexes. Furthermore, we identified by native PAGE, in-gel activity and mass spectrometry this interaction between NDH2e with complexes III and IV in the wild type. Also, larger supercomplexes5 and a complex V dimer were found. This association pattern seems to vary during the stationary phase as NDH2e is overproduced saturating its binding site in Cyt IV and thus appearing as the free enzyme.

3816-Pos

Halophilic Properties of Mitochondria from the Salt-Tolerant Yeast Debaryomyces hansenii

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The osmotolerant, oleaginous and metabolically versatile yeast Debaryomyces hansenii is considered a marine organism. Sea water contains 0.6 M Na+ and 10 mM K+; large quantities of these cations permeate into the cytoplasm of D. hansenii. Therefore, proteins and organelles within the cytoplasm have to adapt to high salt concentrations. Given the choice, D. hansenii accumulates K+ instead of Na+ but both cations seem to have the same effects. The effect of high concentrations of K+ or Na+ on isolated mitochondria from D. hansenii was explored. The mitochondrial respiratory chain from D. hansenii contains the canonical respiratory complexes (I, II, III and IV), plus a cyanide-insensitive alternative oxidase and an external flavone-sensitive NADH dehydrogenase type II. As in S. cerevisiae, these mitochondria undergo a phosphate-sensitive permeability transition (PT), although D. hansenii mitochondria require higher phosphate concentrations to avoid PT. In regard to K+ and Na+, and at variance with mitochondria from all other sources known, these monovalent cations promoted closure of the putative mitochondrial unspecific channel (MUC) as evidenced by the K+/Na+-promoted increase in: respiratory control, transmembrane potential and synthesis of ATP. Thus, in D. hansenii mitochondria K+ and Na+ optimize oxidative phosphorylation, providing an explanation for the higher growth efficiency exhibited by this yeast when exposed to saline environments. Thus, we propose that halophilia is conferred to cells at the subcellular level. It is becoming increasingly evident that the functions and the control mechanisms of MUCs might be different depending of the species under study.

3817-Pos

"Structure and Dynamics of the External Stalk of the FoF1-ATP Synthase"

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The FoF1-ATP synthase is the enzyme responsible for the bulk of ATP synthesized in most organisms. Although the structure and mechanism of the enzyme is generally well-understood, some important intricacies remain unclear. One of the questions still heavily discussed concerns the structure and function of the external stalk which consists of two identical or non-identical subunits b in bacteria and photosynthetic organisms.

Making use of structure prediction, de novo modeling, extensive mutagenesis, site-directed spin labeling and ESR spectroscopy, we suggested that the cytosolic, soluble parts of both the E. coli homodimeric b2 as well as