Complex I (NADH:ubiquinone oxidoreductase) is the first enzyme of the respiratory electron transport chain in mitochondria. It catalyses the reduction of ubiquinone by NADH, coupled to the translocation of four protons across the inner-mitochondrial membrane, and it is a significant source of reactive oxygen species, linked to neuromuscular diseases and ageing. In bovine mitochondria complex I comprises 45 different subunits, a flavin mononucleotide at the active site where NADH is oxidised, and eight iron sulphur clusters. This talk will focus on the mechanism of the redox reaction in the enzyme (the mechanism of proton translocation remains unknown). The structure of the hydrophilic domain of complex I from *Thermus thermophilus* has provided a framework for understanding the redox reaction, and it makes the pathway that the electrons take through the enzyme easy to visualise. However, it does not identify rate limiting steps, or describe the reaction intermediates formed and the free energy changes as the reaction progresses. A mechanistic understanding at this level is crucial for understanding how complex I conserves the potential difference between NADH and ubiquinone as a proton motive force so effectively, for defining how electron and proton transfer are coupled, and for understanding the formation of reactive oxygen species. This talk will describe current understanding and new information about the mechanism of the redox reaction in complex I.

doi:10.1016/j.bbabio.2008.05.020

P/9 Structural and functional insight into mitochondrial complex I

Ulrich Brandt
Johann Wolfgang Goethe-Universität, Fachbereich Medizin, Zentrum der Biologischen Chemie, Molekulare Bioenergetik, Frankfurt am Main, Germany
E-mail: brandt@zbc.kgu.de

The molecular mechanism how complex I (NADH:ubiquinone oxidoreductase) links electron transfer to proton translocation is still elusive. We have established the strictly aerobic yeast *Yarrowia lipolytica* as a powerful genetic system to study structure, function and biogenesis of this mitochondrial complex I. The ubiquinone reducing catalytic core of complex I resides 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. In an extensive mutagenesis study based on the recently published partial structure of bacterial complex I, we have identified the entry pathway for ubiquinone and domains interacting with hydrophobic complex I. Single particle analysis of antibody decorated complex I indicated that the 49-kDa subunit is located surprisingly far away from the membrane arm. This unexpected result was confirmed by further structural studies with a subcomplex of complex I lacking the 51-kDa and 24-kDa subunits. Electron microscopic 3D reconstructions of this subcomplex allowed positioning the partial structure of the bacterial complex within *Y. lipolytica* complex I. Therefore, we propose that ubiquinone reaches its site of reduction via a hydrophobic ramp or channel within complex I. We propose a two-state mechanism of energy conservation for complex I that is based on long range conformational changes of the enzyme driven by stabilization changes of ubiquinone intermediates.

doi:10.1016/j.bbabio.2008.05.021

P/10 The genetics and pathophysiology of mitochondrial disease

Douglas C. Wallace
Center for Molecular and Mitochondrial Medicine and Genetics, University of California, Irvine, Irvine, CA 92697-3940, USA
E-mail: dwallace@uci.edu

Mitochondrial diseases are genetically and phenotypically heterogeneous. The human mitochondrial DNA (mtDNA) $tRNA^{Leu(UUR)}$ G3243A mutation causes diabetes at low heteroplasmy but MELAS at high heteroplasmy, the two phenotypes associated with differences in nuclear DNA (nDNA) and mtDNA gene MITOCHIP gene expression profiles. Inactivation of the mouse heart–muscle–brain isoform of the adenine nucleotide translocator (Ant1) results in mitochondrial myopathy and cardiomyopathy associated with defects in mitochondrial ADP–ATP exchange and increased mitochondrial reactive oxygen species (ROS) production. MITOCHIP expression analysis of Ant1-deficient skeletal muscle revealed the coordinate up-regulation of mtDNA and nDNA oxidative phosphorylation, antioxidant and anti-apoptotic genes and the down-regulation of glycolytic and pro-apoptotic genes, all in association with increased protein levels for Pgc-1α, Nrf1, myogenin, and Tfam but reduced levels of c-myc. Neurons from Ant1-deficient mice were more resistant to glutamate and etoposide induced apoptosis and Ant1-deficient mice were less sensitive to kainic acid excitotoxicity. Mice harboring a heteroplasmic mtDNA ND6 frameshift mutation (nt 13885insC) showed directional loss of the mutant mtDNAs from the female germline in subsequent liters and generations implying intra-ovarian selection against severely deleterious mtDNA mutations. By contrast, a COI nt T6589C missence mutation (V421A) was retained and resulted in the development of mitochondrial myopathy and cardiomyopathy and neuronal cell loss. Therefore, mutations in different mitochondrial genes can produce similar tissue phenotypes, perhaps reflecting similar gene expression profiles.

doi:10.1016/j.bbabio.2008.05.022

P/11 Quantification of the electrochemical proton gradient and activation of ATP synthase in leaves

Pierre Joliot
Institut de Biologie Physico-Chimique, Paris, France
E-mail: Pierre.Joliot@ibpc.fr

We have developed a new method to quantify the transmembrane electrochemical proton gradient present in chloroplasts of dark-adapted leaves. When a leaf is illuminated by a short pulse of intense light, we observed that the light-induced membrane potential change reaches a maximum value (~190 mV) determined by ion leaks that occur above a threshold level of the electrochemical proton gradient. After the light-pulse, the decay of the membrane potential displays a marked slowdown, which reflects the switch from an activated to an inactivated state of the ATP synthase that occurs at ~110 mV. We have estimated the $\tilde{\Delta}\mu_H^+$ level that preexists in the dark (40 to 70 mV), which collapses upon addition of inhibitors of the respiratory chain. Thus, it shows that it results from the hydrolysis of ATP of mitochondrial origin. Illumination of the leaf induces a $\tilde{\Delta}\mu_H^+$ increase (up to ~150 mV) that reflects the light-induced increase in ATP concentration. Following the illumination, $\tilde{\Delta}\mu_H^+$ relaxes to its dark-adapted value according to a multiphasic kinetics completed in more than 1 h. In mature leaf, the deactivation of the Benson–Calvin cycle follows similar kinetics as $\tilde{\Delta}\mu_H^+$ decay, showing that its state of activation is mainly controlled by ATP concentration.

doi:10.1016/j.bbabio.2008.05.023