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The aim of this study was to clarify structures and properties of protozoan parasite Complex II, which often plays a pivotal role in adaptation to hypoxic host environments. Generally, protist mitochondrial enzymes are difficult to purify and still remain uncharacterized. We isolated Complex II from epimastigotes of *Trypanosoma cruzi*, the causative agent of Chagas disease, and identified all genes coded for subunits. In contrast to the mammalian enzyme (SDH1–SDH4), *T. cruzi* Complex II is consisted of six each of hydrophilic (SDH1, $SDH2_N$, $SDH2_C$, and SDH5~7) and hydrophobic subunits (SDH3, SDH4, and SDH8~11). Notably, an iron–sulfur subunit is heterodimeric; $SDH2_N$ and $SDH2_C$ contain the plant-type ferredoxin domain in the *N*-terminal half and the bacterial ferredoxin domain in the *C*-terminal half, respectively. This is a first direct evidence for the splitted SDH2 in Complex II. Sequence analysis indicates that trypanosomatid-specific subunits have evolved by gene duplication of canonical subunits followed by degeneration of one copy. Catalytic subunits contain all key residues for binding of substrates but the enzyme showed the lower affinity for substrates and inhibitors than mammalian enzymes. Further, we characterized Complex IIs from other parasites including malaria parasites, *Ascaris suum* and *Echinococcus multilocularis*. Unusual features of parasite enzymes make Complex II a target for new chemotherapeutic agents.

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S13.27 Fidelity of water-gated mechanism in cytochrome *c* oxidase

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Cytochrome *c* oxidase utilizes the energy released by oxygen reduction to drive proton pumping across the mitochondrial or bacterial membrane. Proton-pumping must therefore be controlled to transfer protons both to a pump site, and to the site for oxygen reduction. Previously, we suggested a mechanism in which water molecules in the non-polar cavity above Glu-242 orientate in a redox state-dependent way to connect Glu-242 either with the D-propionate of heme a_3, or with the oxygenous ligand of Cu_B. To control proton pumping in this way, the energy for the "wrong" orientation of the water molecules must be considerable. We have studied the fidelity of the water-gated mechanism by classical free-energy calculations and molecular dynamics simulations, and explored the cause and energetics of the redox-state dependent orientation of the water molecules.

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S13.28 *c*-type cytochromes coupled to chlorate reduction in *Ideonella dechloratans*

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The present work is part of an investigation of the enzyme chemistry of the chlorate reduction in *Ideonella dechloratans,* a bacterium capable of using chlorate as the sole electron acceptor under anaerobic conditions. The aim of the present work is to investigate the electron transfer route for the chlorate reduction, and to isolate the electron carrier, responsible for the delivery of electrons from the membrane-bound electron transport chain to the periplasmic chlorate reductase. Optical difference spectrum of dithionite reduced periplasm show that at least one of the soluble *c*-type cytochrome can deliver electrons to the periplasmic chlorate reductase. Five native heme-containing proteins, with molecular weights in the range 4.5–20 kDa have been isolated from the periplasm of *I. dechloratans* and identified by SDS-PAGE with heme staining. Two of these (6- and 10-kDa) were purified by IEX and tested as electron donors by optical spectroscopy analysis. After reduction with dithionite, the 6-kDa *c*-cytochrome could be reoxidized by addition of chlorate, in presence of a catalytic amount of chlorate reductase and in absent of oxygen. The 10-kDa cytochrome could not deliver electrons in anaerobic respiration. Both can donate electrons to the terminal cytochrome *c* oxidase when oxygen is present. It is not clear whether the unpurified *c*-cytochromes participate in the electron transfer between the membrane-bound respiratory chain and the periplasmic chlorate reductase.

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S13.29 Purification, characterisation and crystallisation of *Thermus thermophilus* succinate dehydrogenase

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Succinate-ubiquinone oxidoreductase (SQR, Complex II) is unique in being a member of the Krebs TCA cycle as well as the respiratory chain, catalysing the oxidation of succinate to fumarate in prokaryotic and eukaryotic organisms. Recently, we have succeeded in identifying, purifying and characterising succinate dehydrogenase from the extreme thermophilic bacterium *Thermus thermophilus*. *Thermus* complex II differs from mitochondrial complex II as it contains two hemes bound to its transmembrane subunits. The purity and integrity of the enzyme were determined by biochemical and biophysical methods including: UV–Vis spectroscopy, SDS-PAGE, N-terminal sequencing and mass spectrometry. Activity assay and EPR measurements were also performed to measure activity of the purified enzyme and to analyse its redox centres. Crystals of the enzyme have been produced by both the *in surfo* sitting drop and *in meso* crystallisation methods. Optimisation of the crystallisation conditions is underway with a final outlook towards high quality diffracting crystals for X-ray analysis.

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S13.30 The *Escherichia coli* hydrogenase activity under glycerol fermentation

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Escherichia coli catalyses oxidation of formate to H_2 and CO_2 under anaerobic conditions upon fermentation of glucose by formate hydrogen lyase; the latter is suggested to be composed of hydrogenase 4 at neutral or alkaline pH and hydrogenase 3 at acidic pH. *E. coli* has been shown to be also able to ferment glycerol and to produce H_2 at pH 6.5, however terminal pathways and the end products including

the production of H_2 are unclear. At bacterial growth under anaerobic conditions at pH 7.5 and upon fermentation of glycerol, redox potential shift down to ~−650 mV was observed. Using a pair of platinum and titanium-silicate electrodes and other methods, H_2 production activity upon adding glycerol was determined with BW25113, wild type cells. This was increased in *fhlA* and significantly (~3-fold) in *hycE* and *hyfG* mutants but suppressed in *hyaB hybC* mutant. Besides, similar data were obtained upon adding glucose.The results indicate that H_2 can be produced by hydrogenases 1and 2 but not 3 or 4, all of which could function in reverse mode upon glycerol fermentation; pathways and mechanisms should be further studied.

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S13.32 Methylotrophic yeasts as model organisms to study complex I

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Respiratory complex I conveys electrons from NADH in the mitochondrial matrix to ubiquinone in the inner membrane, and pumps protons across the membrane. Complex I isolated from bovine heart mitochondria contains 45 dissimilar subunits, and is the most extensively characterised eukaryotic complex I. The classical eukaryotic model organism *Saccharomyces cerevisiae* does not contain complex I, but it is present in, and has been isolated from, the ascomycetous fungi *Neurosporra crassa* and *Yarrowia lipolytica*. In these cases 39 and 37 different subunits have been identified respectively, and many of these subunits are closely related to those from the mammalian enzyme. Other model eukaryotic species should provide alternative organisms for structural and functional studies of complex I which can exploit site directed mutagenesis, and which may also help us to understand the evolution of this remarkably conserved machinery. Here, we describe the isolation and characterisation of inhibitor-sensitive complex I from the methylotrophic yeast *Pichia pastoris*, previously reported to exhibit no rotenone-sensitive respiration. MALDI and TOF–TOF mass spectrometry were used to identify the major subunits present by their homology to sequences in available databases, and EPR spectroscopy was used to demonstrate the presence of four iron–sulfur clusters, which match well to N1b, N2, N3 and N4 from *Y. lipolytica*. Corresponding results from the related species, *Hansenula polymorpha* are also described.

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S13.33 Does cytochrome *c* oxidase pump protons in the controlled state?

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We are developing a model for cytochrome *c* oxidase activity and its control by electrochemical proton gradients. Mitochondrial or proteoliposomal oxidase turnover is partially inhibited in the presence of a pH gradient or membrane potential. An earlier version of the model assumed that the controlled activity equalled the passive proton return rate, and that the enzyme continued to move charge and pump protons. Measurements of proton permeability however indicate that passive proton return is too slow to account for the controlled respiration rate. The latter can also be modulated by zinc as an inhibitor and by fatty acids as activators under conditions in which passive proton movement is unaffected. Some enzyme mutants can generate a membrane potential and/or pump protons with respiratory control characteristics very different from those of the wild type. Certain bacterial oxidases, such as cytochrome ba_3, exhibit much greater respiratory control than does the mitochondrial enzyme. A model accounting for these features requires that proton return involve the oxidase itself rather than the phospholipid membrane and be an active rather than a passive process (and thus distinct from the classical phenomenon of "slip"). Even the 'chemical' protons needed in the controlled state are then recruited from the outside (P face) of the membrane. In this model the controlled enzyme neither moves charge nor pumps protons yet continues to reduce oxygen.

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S13.34 Structural and functional characterization of *Aquifex aeolicus* sulfide:quinone reductase

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The sulfide:quinone reductase (SQR) is a family of proteins phylogenetically belonging to the glutathione reductase superfamily of flavoproteins. Unlike the other members, though, it is reported to be membrane bound with an unclear topology and fold. It is known to be involved in bacterial and eukaryotic sulfide detoxification and, for some organisms, in the cellular energy production. The aim of the present work is to characterize the 3D structure of *Aquifex aeolicus* SQR. The protein was identified in *A. aeolicus* native membrane preparation by peptide mass fingerprint and purified in presence of detergent by conventional chromatography. It is monodisperse in a dimeric state. The enzyme is active and reveals prolonged thermal stability. Its affinity to Na_2S and to decylubiquinone is in the micromolar range and the quinone analogue antimycin acts as inhibitor. The protein could be crystallized by hanging and sitting drop vapour diffusion, under oil and in sponge phase at 18 °C. The best crystals diffract to 2.40 Å resolution. Experimental phases are currently determined by the MIRAS method, as all attempts to solve the structure by molecular replacement failed. The sites of Os and Au have already been identified and preliminary electron density maps can be calculated at low resolution. Phase extension and model building are underway.

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S13.35 Role of conserved residues of the membrane subunit nuoM in energy conversion by the proton-pumping nadh:ubiquinone oxidoreductase (Complex I)

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Site-directed mutations of the conserved amino acid residues E144, K234, K265 and W342 were introduced into the chromosomal gene /nuoM/ encoding one of the subunits of the membrane domain of /*Escherichia coli*/ Complex. None of the mutated strains has wild type phenotype. The enzyme was expressed in all mutants. Mutated Complex I was isolated and characterized. The quinone reductase