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.

doi:10.1016/j.bbabio.2008.05.374

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.

doi:10.1016/j.bbabio.2008.05.375

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.

doi:10.1016/j.bbabio.2008.05.376

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.

doi:10.1016/j.bbabio.2008.05.377

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