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

activity was practically lost in K234A, K234R and E144A, decreased in W243A and K265A but unchanged in E144D. Complex I from all these mutants contained one mole of tightly bound ubiquinone per mole FMN like wild type enzyme. Estimation of proton-pumping efficiency suggested that the mutant enzymes E144D, W342A and K265A have normal pumping efficiency. Analysis of the amino acid sequences of subunits NuoM and NuoN revealed a clear common pattern, including two lysines that are predicted to be located within the membrane, and which are important for quinone reductase activity. Remarkably, the subunits NuoL and NuoH in the membrane domain also appear to contain conserved lysine residues in transmembrane helices, which may give a clue to the mechanism of proton translocation. A tentative principle of proton translocation by Complex I is suggested based on electrostatic interactions of lysines located in the membrane subunits.

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S13.36 Electrostatic interactions between FeS clusters in Complex I from *Escherichia coli*

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The redox properties of the electron transport chain cofactors of complex I were investigated by spectroelectrochemical potentiometric redox titrations of purified complex I from *E. coli* by means of EPR and optical spectroscopy. The FMN cofactor had a midpoint redox potential (*E*m)~350 mV, (*n*=2). All iron–sulfur clusters can be separated into two groups based on their redox properties, either having a single, *n*=1, or a more complex redox titration curve. The binuclear N1a cluster was titrated with a single (*n*=1) transition, and *E*m ~−235 mV. In contrast, the titration of N1b can only be fitted with the sum of at least two one-electron Nernstians with *E*m values of −245 and −320 mV. The titration curves of the EPR bands attributed to the tetranuclear clusters N2 and N6b can be presented by the sum of at least two components, with *E*m ~−200/−300 mV and −235/−315 mV, respectively. Titrations of the signals from other tetranuclear clusters followed Nernstian *n*=1 curves. The observed redox titration curves are discussed in terms of intrinsic electrostatic interactions between FeS centers in complex I.

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S13.37 A sperm whale myoglobin as protein model of cytochrome a_3: The role of heme propionates

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The propionate carboxylate groups of heme proteins are assigned three roles as: (1) electrostatic anchoring points that help hold the heme in place; (2) a conduit directly participating in heme enzyme electron transfer reactions and (3) a part of proton translocation pathway in respiratory oxidases. In bovine cytochrome *c* oxidase the C- and D-ring propionates of hemes a_3 interact with highly conserved Arg438 and His368 side chains. Similar electrostatic interactions of the heme-6-propionate with Arg45 and the heme-7-propionate with His97 occur at the solvent surface of a sperm whale myoglobin (Mb). This similarity of hydrogen bonding in both proteins suggests that recombinant Mb could be used as a mimic of cytochrome a_3. To investigate the role of propionates we used site directed mutants of oxidized wild type sperm whale Mb, Mb reconstituted with protohemin IX dimethyl ester and also myoglobin prepared by reconstitution of purified heme *a* into apoMb. We have found that the pK_a values of both distal histidine (His64) and water coordinated to ferric heme exhibit a linear dependence on the net charge of the residue at position 45 and heme propionates. Supported by National Institutes of Health Grants GM 35649, HL47020 and Robert A. Welch Foundation Grant C-612.

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S13.38 A vicious cycle-mitochondrial dysfunction leads to beta-amyloid accumulation

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Increasing evidence suggest an important role of mitochondrial dysfunction in the pathogenesis of familial and sporadic Alzheimer's disease (AD). Our stably transfected HEK cell model of familial AD (Amyloid Precursor Protein with Swedish mutation K670M/N671L; APPsw) shows an elevated amount of beta Amyloid (Aβ). This is caused by an increased activity of APP-beta secretase leading to an enormous amyloidogenic processing of APP. APPsw HEK cells are characterized by higher reactive oxygen species (ROS) production, lowered mitochondrial membrane potential, decreased ATP-levels and reduced NADH/NADPH-related redox activity compared to untransfected HEK cells. These data demonstrate the mitochondrial toxicity of Aβ. In this study, we addressed the question if mitochondrial dysfunction itself induces Aβ production. We incubated untransfected HEK cells with complexes inhibitors of the mitochondrial respiratory chain and found increased Aβ production after inhibition of the complexes I, II and III, which are known to play an important role in generating ROS. Exposure to ROS also increased Aβ level. Therefore, we propose that mitochondrial dysfunction itself induces Aβ generation mediated by ROS. This could be an important pathomechanism for sporadic as well as for familial AD. In sporadic AD, mitochondrial deficits and age-associated increase in ROS levels could be the initiative for elevated Aβ production. In familial AD, Aβ itself could influence the function of the respiratory chain complexes, increasing ROS levels and hence accelerating its own production.

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S13.39 Mitochondrial dysfunction in Tau-SY5Y cells — A model for Alzheimer's disease and FTDP-17

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Neurofibrillary tangles (NFT) are abundant in many neurodegenerative diseases, including Alzheimer's disease (AD). NFTs are composed of paired helical filaments (PHFs) made of hyperphosphorylated tau. Mutations in the tau gene lead to hyperphosphorylation and loss of physiological function. As mitochondrial dysfunction plays an important role in neurodegenerative disorders, we examined the chronic