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