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.

doi:10.1016/j.bbabio.2008.05.378

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.

doi:10.1016/j.bbabio.2008.05.379

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.

doi:10.1016/j.bbabio.2008.05.380

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.

doi:10.1016/j.bbabio.2008.05.381

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

impairment of over expression of the longest tau isoform (hTau40) and mutant tau (TauP301L) on mitochondrial function in SH-SY5Y cells. Additionally, we tested the influence of inhibitors of the mitochondrial respiratory chain complexes, as in human P301L FTDP17 brains the level of complex V is reduced. We found that over expression of human wtTau or TauP301L leads to mitochondrial dysfunction in our cell model. Already under basal conditions the ATP level and the metabolic activity are significant decreased in TauP301L cells compared to hTau40 cells. Additional stress with the complex inhibitors results in a dose-dependent loss of metabolic activity, reduced ATP levels and depolarized MMP in all three cell types.

doi:10.1016/j.bbabio.2008.05.382

S13.40 Characterization of the redox centres in arsenite oxidase

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Arsenic is a toxic element, present in water predominantly as As^{V} and As^{III}. Microorganisms strongly affect its speciation in the environment, for example by converting As^{III} to As^{V} via their arsenite oxidase, thereby detoxifying their growth medium. The crystal structure of the *Alcaligenes faecalis* enzyme revealed a linear arrangement of its three redox centres, suggesting a linear electron transfer from Molybdenum to a [3Fe–4S] and on to a Rieske-type [2Fe–2S] centre. An electrochemical study determined an $n=2$ redox transition for the Mo atom and E_m values for the three centres rendering the proposed electron transfer thermodynamically unfavourable at pH 6. We have recently addressed this question using i) another enzyme, that from NT-26, ii) another experimental approach, *i.e.* EPR and iii) a pH-screening between 6 and 9.5. As already established for mesophilic cytochrome *bc*-Rieskes, the E_m of the Rieske centre remains constant (at 220±10 mV) up to pH 8 and decreases above pH8 with a slope of −80 mV/pH. In this pH range, the redox state of the [3Fe–4S] centre was unstable even at cryogenic temperatures. Titrating the [3Fe–4S] centre was only possible in the presence of sulphite (shown to be an inhibitor of arsenite oxidase) yielding $E_{m6}=+260$ mV, *i.e.* close to that reported for *Alcaligenes*. We interpret these observations as reflecting a redox re-equilibration between the [3Fe–4S]- and the Mo-centres. The results are discussed to propose an electron flow model through the enzyme.

doi:10.1016/j.bbabio.2008.05.383

S13.41 Bamboo mitochondrial energy metabolic pathways in *Bambusa oldhamii* and *Phyllostachys edulis* during rapid shooting stage

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The energy-conversing and energy-dissipating systems were studied in young bamboo shoot mitochondria isolated from summer bamboo *Bambusa oldhamii* and from winter bamboo *Phyllostachys edulis*. The mitochondrial respiration rates of NADH, succinate or malate oxidation were measured at 15, 28, and 42 °C. Temperature raised from 15 °C to 28 °C, the increased respiration rate of *P. edulis* were higher than that in *B. oldhamii*, whereas the temperature raised from 28 °C to 42 °C, the increased respiration rate of *P. edulis* were lower than those of *B. oldhamii*. The calculated Q_{10} values of *B. oldhamii* at intervals of 15~28 °C and 28–42 °C were about 1.9–2.4 and different from those of *P. edulis*. Moreover, the membrane thermostability of *B. oldhamii* mitochondria was suggested to be lower than of *P. edulis* as the critical temperature of *B. oldhamii* was about 20 °C and that of *P. edulis* mitochondria about 25 °C. Furthermore, alternative oxidase (AOX), plant uncoupling mitochondrial protein (PUMP), and plant mitochondrial potassium channel ($PmitoK_{ATP}$) were investigated. In the presence of SHAM, an AOX inhibitor, more than 50% of the respiration rate was inhibited in *B. oldhamii* whereas only a small portion of 6.9% respiration in *P. edulis* was inhibited. In the presence of PUMP activator linoleic acid, mitochondrial membrane potential was collapsed about 85% in *P. edulis* and 30% in *B. oldhamii*. It showed that the activity of $PmitoK_{ATP}$ in *P. edulis* mitochondria was probably 2 folds of that in *B. oldhamii* as a rapid swelling occurred in *P. edulis* with addition of KCl whereas a mild swelling occurred in *B. oldhamii*. The results may support that *P. edulis* adapting to chilling environment was correlated to higher energy-dissipating capacity than *B. oldhamii* favoring to moderate environment.

doi:10.1016/j.bbabio.2008.05.384

S13.42 Alternative oxidase 1a in *Arabidopsis thaliana* is required for normal stress response

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The aim of this study was to determine the function of the alternative oxidase in *Arabidopsis*. Treatment of *alternative oxidase 1a* mutant plants (*aox1a*) with moderate light and drought resulted in changes is respiration, photosynthesis, reactive oxygen species and metabolites that were absent or much less pronounced in Col-0 plants. These changes were accompanied by drastic changes in the transcriptome during the stress treatment, affecting genes encoding proteins involved in a wide variety of processes in various locations in the cell. Functional analysis of the *AOX1a* promoter revealed that it contain *cis*-acting regulatory elements previously identified to be involved in stress responses in a variety of genes, in particular stress responses mediated by abscisic acid. These results indicate that AOX1a is required for a normal stress response in *Arabidopsis* and its regulation interacts with mainstream stress signalling pathways.

doi:10.1016/j.bbabio.2008.05.385

S13.43 Inhibitors of succinate dehydrogenase (SDH) and complex III promote respiration of liver mitochondria under conditions of functioning DT-Diaphorase (DTD)

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Mitochondrial complex III interacts with three dehydrogenises. Two of them complex I and DTD oxidize NADH, SDH oxidizes succinate. Both NADH and succinate are synthesized in Krebs cycle. The competition of DTD and SDH in course of their interaction with bc_1-complex was investigated. All measurements were carried out with malate in the capacity of respiration substrate. Complex I was inhibited by rotenone. Duroquinone or CoQ_0 was taken as a second substrate of DTD. On the one hand we found out that low concentrations of Q-cycle o-center inhibitor myxothiazol under conditions of functioning DTD initiate small stimulation of respiration. And on the other hand inhi-