plays a critical role in human muscle diseases *in vivo*; and they represent an important proof of principle that hereditary muscle diseases can be cured with proper drugs downstream of the genetic lesion if the pathogenetic mechanisms are understood. This is a useful example of how translational medicine can rapidly move from animal models to treatment of human diseases; and of how mitochondrial medicine may be useful beyond the cure of primary mitochondrial diseases.

doi:10.1016/j.bbabio.2008.05.035

P/24 Distance metrics for heme protein electron tunneling

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Distance is the principal parameter that sets the order of magnitude for electron-tunneling rates; however, there continue to be varying ways to measure electron-tunneling distances in proteins. This distance uncertainty blurs the issue of whether the intervening protein medium has been naturally selected to speed or slow any particular electron-tunneling reaction. For redox cofactors lacking metals, or for chlorophylls, distances can be defined from conjugated cofactor edge atoms, approximating the space that includes most of the tunneling electron wavefunction. However, for the iron-containing heme, an appropriate cofactor edge is ambiguous. Electron-tunneling distances may be measured from the conjugated heme macrocycle edge or from the metal, which can be up to a significant 4.8 Å longer. To address this ambiguity, we consider both natural heme protein electron transfer and light-activated electron transfer in ruthenated heme proteins. We find that the edge of the conjugated heme macrocycle provides a reliable and useful tunneling distance definition consistent with other biological electron-tunneling reactions. Furthermore, with this distance metric, heme axially- and edge-oriented electron transfers appear similar and are equally well described by a simple square barrier tunneling model. This is in contrast to recent reports for metal-to-metal metrics that require exceptionally poor donor/acceptor couplings to explain heme axially-oriented electron transfers.

doi:10.1016/j.bbabio.2008.05.036

P/25 Is the modified Q-cycle sufficient as a model to describe the mechanism of the bc_1 complex without invoking electron transfer across the dimer interface?

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Recent progress in understanding the Q-cycle mechanism of the bc_1 complex is reviewed. The data strongly support a mechanism in which the Q_o-site operates through a reaction in which the first electron transfer from ubiquinol to the oxidized iron–sulfur protein is the rate determining step for the overall process. The reaction involves a proton-coupled electron transfer down a hydrogen bond between the ubiquinol and a histidine ligand of the [sFe-2S] cluster, in which the unfavorable protonic configuration contributes a substantial part of the activation barrier. The reaction is endergonic, and the products are an unstable ubisemiquinone bound at the Q_o-site, and the reduced iron–sulfur protein, the extrinsic mobile domain of which is now moves away to deliver an electron to cyt c_1 and liberate the H^+. When oxidation of the semiquinone is prevented, it participates in bypass reactions, including superoxide generation if O_2 is available, which are minimized by the above characteristics, and through coulombic gating. The mechanism discussed is monomeric, but the literature suggests that the dimeric structure participates in a more complicated mechanism. We show from myxothiazol titrations and mutational analysis of cyt *b* Tyr-199, that no inter-monomer electron transfer occurs at the level of the b_L hemes. Analysis of strains with mutations at Asn-221 shows that there are coulombic interactions between the b-hemes in a monomer, and likely also across the dimer interface.

doi:10.1016/j.bbabio.2008.05.037

P/26 The systems biology of the mammalian mitochondria

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The mitochondrion is a self contained biochemical machine involved numerous functions of the cell. A quantitative mathematical model of mitochondria function reveals deficits in our understanding of this process including the enzyme catalyzed reaction pathways and kinetics, post-translational modifications and chemical activity of reaction intermediates. To begin to fill these gaps, the tissue specific mitochondrial biochemical pathways have been characterized using quantitative proteomics by several groups. Using this data, the nuclear "protein program" for a mitochondrial function was evaluated. An example of "reprogramming" associated with Type I diabetes will be discussed. With this metabolic roadmap, the acute regulation of enzymatic function was evaluated by screening matrix phosphoproteome using fluorescent probes, isoelectric focusing and ^{32}P labeling. These studies reveal an extensive and dynamic matrix phosphoproteome. The functional significance, specific interaction sites and nature of the matrix kinase/phosphatase system, including autophosphorylation, are being investigated. All of these approaches, along with those from many other laboratories, begin to fill in the gaps in a quantitative consensus model of mitochondrial function. Clearly this type of systematic approach will need to be continued to establish the entire mitochondria metabolic reaction network and its regulation.

doi:10.1016/j.bbabio.2008.05.038

(S1) ATP synthase/ATPase symposium lecture abstracts

S1/1 Mode of ion binding in the ATP synthase family

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ATP synthases operating in tandem with a respiration or light-driven proton pump use protons and those operating in tandem with a

decarboxylation-driven Na+ pump use Na+ as the coupling ion. Specific binding sites for the respective coupling ion are present in the oligomeric ring of c subunits. In the c ring from *Ilyobacter tartaricus*, each sodium ion is coordinated by side chain and backbone oxygens from the inner and outer helices of two neighboring c subunits and the binding pocket is firmly stabilized by hydrogen bonds donated to the conserved cE65residue. In this conformation the sodium ion is buried and cannot reach putative ion conducting channels in subunit a. Cysteine–cysteine cross-linking studies between subunits a and c indicated subtle but distinct conformational changes around the Na+ binding site that were elicited by the stator arginine. These experiments support a model in which the alkali ion is released from the binding site by adapting a conformation that favors arginine but not Na+ binding. pH profiles for DCCD inhibitor binding by the proton translocating ATP synthase of *Halobacterium salinarium* reflected the popular group protonation mechanism of the conserved carboxylates in the c ring. However, all other ATP synthases investigated yielded pH profiles that could be best explained by the coordination of a hydronium ion. Hence, three different modes of ion binding (Na+, H3O+, H+) have developed in different ATP synthases during evolution.

doi:10.1016/j.bbabio.2008.05.039

S1/2 Mechanistic insights of F_1-ATPase rotation from single-molecule measurements of the power stroke

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Single-molecule measurements of the rotation of the F_1-ATPase γ subunit from *E. coli* were made with a time resolution that allowed the power stroke (transition between dwells) to be resolved. The duration of transitions and dwells was measured as a function of the viscosity of the medium using PEG-400 with various sizes of gold nanorods attached to the γ subunit as a visible probe of rotation. In the absence of PEG, the power stroke was found to be viscous-limited when nanorods with dimensions of 91×45 nm were used as probes, but was kinetically-limited when 75×35 nm, 87×36 nm, and 90×46 nm were used. The dwell times matched ATPase turnover rates measured in bulk solution without an attached nanorod at low viscosities, and increased about 8 fold as a function of viscosity under conditions in which the power stroke was kinetically limited. When the power stroke became limited by the viscous drag, the dwell times became longer than the ATPase turnover time in the absence of a bound nanorod. Under these conditions, the increase in the transition time caused a 20 fold increase in the dwell time regardless of the size of the nanorod used. These results indicate that forcing the γ subunit to rotate more slowly than occurs by the intrinsic kinetically-limited mechanism causes the enzyme mechanism to deviate from its normal catalytic cycle, and provide insight into sequential conformational states of the enzyme during a catalytic cycle.

doi:10.1016/j.bbabio.2008.05.040

S1/3 The stator stalk of *Escherichia coli* ATP synthase

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The peripheral stator stalk of *E. coli* ATP synthase is formed by a dimer of helical, 156-residue *b* subunits that are anchored in the membrane by N-terminal transmembrane segments. b_2 reaches up the side of F_1 and binds near the top, interacting with the δ subunit. Most of this distance is spanned by the dimerization domain contained within residues 53–122. Sequence analysis reveals an unusual, alanine-rich 11-residue repeat pattern, consistent with a novel, two-stranded right-handed coiled coil (RHCC) structure. Disulfide formation studies, and analysis of the shapes and stabilities of disulfide-linked forms, support an RHCC structure with helices offset by 5–6 residues, making the structure intrinsically asymmetric. The RHCC is controversial and a left-handed coiled coil has also been proposed. Chimeric forms of *b* incorporating exogenous sequences containing a hendecad pattern similar to that of *b* into the region between positions 55 and 95 supported ATP synthesis *in vivo*, but those incorporating known left-handed coiled coil sequences failed to do so, even though ATP synthase still assembled. Single residue deletions within the dimerization domain also support assembly but not ATP synthesis, confirming that the stator stalk has a functional role beyond simply holding on to F_1. The significance of the proposed offset RHCC structure in binding to F_1 and resisting rotational torque will be discussed.

doi:10.1016/j.bbabio.2008.05.041

S1/4 Domain compliance and elastic power transmission in F_OF_1-ATPase

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F_OF_1-ATP synthase is composed from two stepping rotary motors coupled by a common rotary shaft. The electromotor, F_O, drives the chemical generator, F_1. Elastic power transmission between F_O is indispensible for their coupled operation at high speed. By fluctuation analysis the torsional spring constants of various enzyme domains were determined, engineered SS-bridges served to selectively stiffen others. Both, the central shaft in F_1 and, surprisingly, also the long eccentric bearing, were rather rigid. Only one domain of the rotor, namely where subunits γ and ε of F_1 contact the c-ring of F_O, was more flexible (50 pNnm) by order of magnitude. This elastic buffer, being located between the loci of torque delivery by F_O and by F_1, provides high kinetic efficiency to this twin engine under load, and it accounts for the ability of concerted action with different gears in different organisms.

doi:10.1016/j.bbabio.2008.05.042

S1/5 Photoaffinity labeling and photoaffinity cross-linking of ATP synthase complexes

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Photoaffinity labeling and photoaffinity cross-linking have been proven to be valuable techniques for the localization and characterization of ligand binding sites. In order to characterize nucleotide binding sites of ATP synthases we have synthesized various mono- and bifunctional photoactivatable ATP analogs. The six nucleotide binding sites – three catalytic and three noncatalytic – of ATP synthases are