at Cu_B in the O-state, or an additional hydroxyl group at Fe_{a3}. However, the electron densities of all high resolution structures are compatible best with a peroxo-bridge between Fe_{a3} and Cu_B. In addition, we find that the F-state generated in the traditional way by an excess of H_2O_2, can be converted into a P-state simply by addition of catalase. Finally we have discovered conditions under which the O-state spontaneously converts into a P-state with concomitant formation of a tyrosine radical. All these results can be understood best if the catalytic cycle starts with an O-state containing a bridging peroxide dianion and the F-state contains a superoxide bound to Cu_B.

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P/20 The proton translocation mechanism of cytochrome *c* oxidase

Marten Wikstrøm
Institute of Biotechnology PB 65 (Viikinkaari 1), FIN-00014 University of Helsinki, Helsinki, Finland
E-mail: marten.wikstrom@helsinki.fi

The cytochrome *c* oxidases (CcO), which are responsible for most of the O_2 consumption in biology, are also redox-linked proton pumps that effectively convert the free energy of O_2 reduction to an electrochemical proton gradient across mitochondrial and bacterial membranes. Recently, time-resolved measurements have elucidated the sequence of events in proton translocation, and shed light on the underlying molecular mechanisms. One crucial property of the proton pump mechanism has received less attention, *viz*. how proton leaks are avoided. Here, we will analyse this topic and demonstrate how the key proton-carrying residue Glu-242 (numbering according to the sequence of subunit I of bovine heart CcO) functions as a valve that has the effect of minimising back-leakage of the pumped proton.

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P/21 The proton pumping heme-copper oxidases

Robert B. Gennis
Department of Biochemistry, University of Illinois, Urbana, IL 61801, USA
E-mail: r-gennis@uiuc.edu

All known proton pumping respiratory oxidases are members of the heme-copper superfamily. This superfamily contains not only the oxygen reductases (respiratory oxidases) but also prokaryotic NO reductases that are used for denitrification and detoxification. Our work has focused on the largest group of respiratory oxidases, the A-family, with the aim being to understand how the chemistry of reducing oxygen to water is coupled to driving a unidirectional proton pump. Most studies have used the aa_3-type oxidase from *R. sphaeroides*. Mutations in one of the two proton input channels, the D channel, can decouple the proton pump from the redox chemistry. The properties of these mutants will be discussed. Recent studies have included respiratory oxidases that are not members of the major (canonical) family of heme-copper oxidases, but are in the B- and C-families. This includes work on the ba_3-type oxidase from *Thermus thermophilus*, in a collaboration with the group of Dr. James Fee (Scripps Institute). Results will be discussed.

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P/22 Cytochrome *c* binding to the cytochrome bc_1 complex: An interaction critical for electron transfer

Sozanne Solmaz[a], Tina Wenz[a], Ajeeta Nyola[a], Carola Hunte[b]
[a]*Max Planck Institute of Biophysics, Frankfurt a.M., Germany*
[b]*University of Leeds, Leeds, UK*
E-mail: c.hunte@leeds.ac.uk

In cellular respiration, the mobile electron carrier cytochrome *c* shuttles electrons from cytochrome bc_1 complex to cytochrome *c* oxidase. The X-ray structure of the complex between cytochrome *c* and cytochrome bc_1 complex at 2.97-Å resolution gave the first structural insight for such a complex from the respiratory chain. The structure revealed the general features of the interface, which is well suited for transient interaction and fast turnover. Remarkably, cytochrome *c* binds to only one recognition site of the homodimeric complex. We now determined the structure of the electron transfer complex in the reduced state at 1.9-Å resolution. The high resolution allows an accurate description of the interface, especially of electrostatic and water-mediated interactions. The dimer structure is asymmetric. Monovalent cytochrome *c* binding is correlated with conformational changes of the Rieske head domain and subunit QCR6p and with a higher number of interfacial water molecules bound to cytochrome c_1. Comparison with a second structure obtained for isoform-2 cytochrome *c* bound to the cytochrome bc_1 complex led to the definition of a minimal interface, the so-called core interface, which is present in all of these structures. The importance of single core interface residues for formation of the reactive complex in solution was probed by site-directed mutagenesis and characterization of the variants.

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P/23 Toward a mitochondrial therapy of collagen VI muscular dystrophies

Paolo Bernardi
Department of Biomedical Sciences, University of Padova, Viale Giuseppe Colombo 3, I-35121 Padova, Italy
E-mail: bernardi@bio.unipd.it

Ullrich Congenital Muscular Dystrophy (UCMD) and Bethlem Myopathy (BM) are muscle diseases due to mutations in the genes encoding the extracellular matrix protein collagen VI. Generation of a dystrophic mouse model where collagen VI synthesis was prevented by genetic ablation of the *Col6a1* gene allowed an investigation of pathogenesis, which revealed the existence of a Ca^{2+}-mediated dysfunction of mitochondria and the sarcoplasmic reticulum. A key event appears to be inappropriate opening of the mitochondrial permeability transition pore, an inner membrane high-conductance channel. Consistently, the *Col6a1*$^{-/-}$ myopathic mice could be cured with cyclosporin A through inhibition of cyclophilin D, a matrix protein that sensitizes the pore to opening. Studies of myoblasts from UCMD and BM patients demonstrated the existence of a latent mitochondrial dysfunction irrespective of the genetic lesion responsible for the lack or the alteration of collagen VI. These studies suggest that PTP opening may represent the final common pathway for skeletal muscle fiber death; and provided a rationale for a pilot clinical trial with cyclosporin A in patients affected by UCMD and BM. Prior to treatment, all patients displayed mitochondrial dysfunction and increased frequency of apoptosis, as determined in muscle biopsies. Both these pathological signs were largely normalized after 1 month of oral cyclosporin A administration, which also increased muscle regeneration. These results indicate that mitochondrial dysfunction

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.

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P/24 Distance metrics for heme protein electron tunneling

Christopher C. Moser, Sarah E. Chobot, Christopher C. Page, P. Leslie Dutton
Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, USA
E-mail: dutton@mail.med.upenn.edu

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.

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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?

A.R. Crofts[a,c], J.T. Holland[c], D. Victoria, D.R.J. Kolling[c], S.A. Dikanov[b], R. Gilbreth[c], S. Lhee[c], R. Kuras[c], M. Guergova Kuras[c]
[a]*Department of Biochemistry, University of Illinois at Urbana-Champaign, USA*
[b]*Department of Veterinary Medicine, University of Illinois at Urbana-Champaign, USA*
[c]*Center for Biophysics and Computational Biology, University of Illinois at Urbana-Champaign, USA*
E-mail: a-crofts@life.ujuc.edu

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.

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P/26 The systems biology of the mammalian mitochondria

Robert Balaban
National Heart, Lung and Blood Institute, Bethesda, USA
E-mail: balabanr@nhlbi.nih.gov

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.

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(S1) ATP synthase/ATPase symposium lecture abstracts

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

Christoph von Ballmoos, Peter Dimroth
Institute of Microbiology, ETH Zurich, Switzerland
E-mail: dimroth@micro.biol.ethz.ch

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