

resolution of the ETC complexes in brain mitochondria. The protein complexes of the mitochondrial oxidative phosphorylation system have been reported to form supramolecular assemblies termed respiratory supercomplexes or respirasomes. BN-PAGE was used in this study to analyze the mitochondrial subunit assembly into respiratory chain complexes in rat brain synaptic and non-synaptic mitochondria. Using the mild detergent digitonin for solubilisation of mitochondrial membranes, it was shown that complexes I and II–V interact to form supercomplexes. However, initial experiments suggest that the supercomplex composition is different between synaptic and non-synaptic mitochondria from rat brain. The consequences for such disparity in supercomplex formation will be discussed.

doi:10.1016/j.bbabi.2008.05.421

S16.7 Analysis of proteins released through the permeability transition pore of rat brain mitochondria

Rashmi U. Pathak, Stephen R. Quinn, Martha Motherway Gildea, Gavin P. Davey

School of Biochemistry and Immunology and Trinity College Institute of Neuroscience, Trinity College Dublin, Ireland

E-mail: upadhyar@tcd.ie

Mitochondrial dysfunction can contribute to cell death by not only energetic failure and increased ROS production, but also by the activation of the mitochondrial permeability transition pore (PTP) and release of proapoptotic proteins. The PTP complex is a dynamic poly-protein complex, which spans both mitochondrial membranes at the contact site. An elevation of matrix calcium, beyond a critical threshold, is one of the strongest inducers of the pro-apoptotic PTP. Differing flux control coefficients and energy thresholds have been recorded between synaptic and nonsynaptic mitochondria extracted from rat brain, however, little is known about the proteins that are released from their respective PTPs under stressful conditions. In this study we investigated the calcium-induced swelling in energized/de-energized synaptic and non-synaptic rat brain mitochondria. We report that rat brain PTP opening (as measured by swelling) in both types of mitochondria was more sensitive to Bongkrekic acid than to Cyclosporin A. Furthermore, following swelling of the mitochondria, the proteins released through the pore were resolved on a 2D-PAGE and identified by MALDI-TOF mass spectrometry. The differences between the proteins released through the PTPs from synaptic and non-synaptic rat brain mitochondria and their physiological implications will be discussed.

doi:10.1016/j.bbabi.2008.05.422

(C1) Genomics and evolution colloquium lecture abstracts

C1/1 Introductory notes: Energetic constraints at the very beginning of life

Armen Y. Mulikidjanian

School of Physics, University of Osnabrueck, Osnabrück, Germany

A.N.Belozersky Institute of Physico-Chemical Biology, Moscow State University, Russia

E-mail: amulid@uos.de

The number of hypothetical scenarios for the origin of life is unlimited. The space of possibilities, however, can be dramatically restricted by consistently invoking physical, chemical, biological and geological constraints. The short introductory talk will focus on energetic constraints, in particular on the consideration of energy

sources that could be available and utilizable at the earliest stages of evolution.

doi:10.1016/j.bbabi.2008.05.423

C1/2 Energetics of the first bacteria as inferred from genome analysis

Michael Y. Galperin^a, Eugene V. Koonin^a, Kira S. Makarova^a, Armen Y. Mulikidjanian^b

^aNCBI, NLM, National Institutes of Health, Bethesda, Maryland, USA

^bSchool of Physics, University of Osnabrück, Osnabrück, Germany

E-mail: galperin@ncbi.nlm.nih.gov

The availability of complete genome sequences had a major impact on modern biology, resulting in a much better understanding of cell metabolism. Owing to their complex subunit structure, membrane energy-transducing complexes remained outside the scope of most comparative-genomic analyses. We compared the distribution of proton- and sodium-translocating enzymes encoded in bacterial and archaeal genomes, analyzed the physico-chemical and evolutionary constraints for their origin, and used these data to infer an evolutionary scenario for the origin of the energy transduction machinery. Surprisingly, results of comparative structural and phylogenetic analyses suggest that sodium-translocating ATP synthases and ion pumps preceded the proton-translocating ATP synthases and proton pumps. Thus, the first prokaryotes likely relied on sodium ion gradient for their energy metabolism. Proton-based energetics must have emerged later, following the development of proton-tight membranes through different adaptations in bacteria, archaea and eukaryotes. Evolutionary advantages of proton-based energetics, in particular, chemical coupling of transmembrane proton translocation with electron transfer from organic substrates to terminal electron acceptors, such as oxygen or nitrate, ensured wide dissemination of the corresponding genes and resulted in the switch from Na⁺ to H⁺ as the coupling ion in most bacteria and archaea. Currently, sodium-based energetics is found primarily in obligate anaerobic prokaryotes, including some important human pathogens.

doi:10.1016/j.bbabi.2008.05.424

C1/3 Chloroplast sensor kinase — The redox messenger of organelle gene expression

John F. Allen, Sujith Puthiyaveetil

School of Biological and Chemical Sciences, Queen Mary, University of London, Mile End Road, London E1 4NS, UK

E-mail: j.f.allen@qmul.ac.uk

Why are there genes in chloroplasts and mitochondria? The CoRR hypothesis states that organellar genes and their gene products are Co-located for Redox Regulation. CoRR predicts (i) that an irreducible core of genes must be retained by chloroplasts and mitochondria from their bacterial ancestors, and (ii) that a bacterial redox signalling pathway exerts regulatory control over expression of these genes, using components that have operated continuously throughout the transition from prokaryote to bioenergetic organelle. Chloroplast Sensor Kinase (CSK) is a chloroplast stromal protein that is the product of the nuclear gene *At1g67840* of *Arabidopsis thaliana*. T-DNA insertion lines are impaired in plastoquinone redox control of transcription of chloroplast genes for reaction centre apoproteins of photosystem I and II and do not adjust PS I/PS II stoichiometry. CSK is homologous with bacterial histidine sensor kinases and yet is universal in photosynthetic eukaryotes. We propose that CSK provides the redox regulation

that alone justifies the huge cost of maintaining a small quasi-autonomous genetic system in the chloroplast.

doi:10.1016/j.bbabbio.2008.05.425

C1/4 Energy and informational fluxes in evolution – The key to complexity in the biosphere

Antony R. Crofts

Department of Biochemistry, University of Illinois at Urbana-Champaign, USA

E-mail: a-crofts@life.uiuc.edu

Two great fluxes control the biosphere, – energy and information. Evolution, behavior, cellular recognition and signaling, and human communication are examples of the latter. Shannon recognized that information transmission required two components, engineering aspects and the semantic content or ‘meaning’ of the message. This semantic component cannot be quantified within the same thermodynamic framework as bioenergetic aspects. The message needs a thermodynamic carrier, but the meaning has value only in context and after translation and interpretation. These characteristics lead to some interesting conclusions about the role of semantic transmission in the development of complexity through evolution, and in human culture. Translation is essential, both mechanistically, and because it allows an increase in combinatorial potential through dimensionality. Exploitation of combinatorial potential is constrained by the evolutionary heritage, so that the biosphere shares a highly restricted informational base, which increases in complexity with time. In humans, complexity is extended to the cultural domain. Genetic and cultural channels for inheritance have different components, but they share a common feature in their need for a semantic component, and matching translational machineries, and this justifies a Universal Darwinism. This leads to a view of the evolutionary process in terms of success in exploitation of thermodynamic potential through an exploration of the nature of reality; – a molecular epistemology encapsulated in the commonality of life.

doi:10.1016/j.bbabbio.2008.05.426

(C2) Controversial issues in cytochrome oxidase colloquium lecture abstracts

C2/1 Controversial issues and conformational changes in cytochrome c oxidase

Shelagh Ferguson-Miller, Ling Qin, Michael Garavito

Biochemistry and Molecular Biology, Michigan State Univ., E. Lansing, MI48824, USA

E-mail: fergus20@msu.edu

Despite high resolution structures and powerful spectral and computational methods for analysis of terminal oxidases, the molecular mechanism remains contentious. Areas at issue include the pathways and key residues involved in proton movement, the timing of proton uptake and release with respect to electron transfer, the role of water clusters as proton acceptors, regulatory mechanisms and the nature of catalytic intermediates. Another subject of conflicting views and data is whether significant conformational changes are involved in the coupling mechanism. Small, localized changes, or no changes in structure upon complete reduction have been reported previously. We have solved the structures of resting, reduced and re-oxidized forms of *Rhodobacter sphaeroides* CcO at 2.0–2.2 Å. The reduced form shows little change in overall structure, but the entire porphyrin ring of heme

a_3 and its hydroxyl-farnesyl tail are shifted 1–3 Å, opening the top of the K proton channel and impacting critical residues in the K path. Also in the K-channel region, a high occupancy binding site for the bile acid deoxycholate is resolved. Bile acids are strong inhibitors of the bovine CcO and a binding site is found in the same location as in RsCcO. The results suggest that redox state-induced conformational change, and a conserved steroid binding site, could regulate proton uptake in the K path. (NIH GM26916; MSU REF03-016).

doi:10.1016/j.bbabbio.2008.05.427

C2/2 Rapid kinetic studies of electron transfer in cytochrome oxidase

Francis Millett^a, Sue E. Brand^a, Sany Rajagukguk^a, Krithika Ganesan^b, Lois Geren^a, Marian Fabian^c, Dan Han^b, Robert B. Gennis^b, Bill Durham^a, Shelagh Ferguson-Miller^d, Denise A. Mills^d

^aUniversity of Arkansas, USA

^bUniversity of Illinois, USA

^cRice University, USA

^dMichigan State University, USA

E-mail: millett@uark.edu

The 1-electron reduction of pulsed, oxidized cytochrome oxidase was investigated using a new photoactive binuclear ruthenium complex, [Ru(bipyrazine)₂]₂(quaterpyridine), (Ru₂Z). The photoexcited state Ru(II*) of Ru₂Z is reduced by aniline to Ru(I), which then reduces Cu_A with yields up to 60%. The pulsed O_H state of cytochrome oxidase was prepared by a stopped-flow-flash technique. Mixing fully reduced anaerobic enzyme with oxygenated buffer containing Ru₂Z resulted in formation of the oxidized O_H state within 5 ms. Ru₂Z was then excited with a laser flash to inject 1 electron into Cu_A. Electron transfer from Cu_A to heme a occurred with a rate constant of 20,000 s⁻¹ in the bovine oxidase, followed by electron transfer from heme a to Cu_B with rate constants of 750 s⁻¹ and 110 s⁻¹ and 63% completion. The extent of electron transfer from heme a to Cu_B was only 30% in the non-pulsed O form, indicated a significant difference between the pulsed O_H and non-pulsed O form of bovine oxidase. In contrast, pulsing did not have a significant effect on electron transfer in *Rhodobacter sphaeroides* cytochrome oxidase. The role of electrostatics in controlling electron transfer in cytochrome oxidase has also been explored.

Supported in part by NIH grants GM20488 and RR15569.

doi:10.1016/j.bbabbio.2008.05.428

C2/3 A redox coupled proton pumping mechanism for the B-type cytochrome c oxidases: Density functional studies of the ba₃-oxidase from *Thermus thermophilus*

James A. Fee, David A. Case, L. Noodleman

Department of Molecular Biology, The Scripps Research Institute, La Jolla, California, USA

E-mail: jafee@scripps.edu

The aim of our work is to derive a mechanism of proton pumping by the heme-copper oxidases. After a brief introduction to structural details of ba₃-oxidase concerning the oxygen-uptake channel, the oxygen-in to water-out event, and the nature of the active-site, Fe_{a3}-Cu_B pair, evidence will be presented to support only one proton uptake path from the inside, namely a modified K-path. The bulk of the talk will focus on Density Functional Theory calculations of an ~200-atom active-site model, and thermochemical deductions therefrom, that support a novel, fourteen-step, chemically-detailed, redox-coupled mechanism for proton-pumping by the B-type oxidases. An explana-