of 2.8 mM glucose. Increased glucose and pyruvate each produced a concentration-dependent mitochondrial hyperpolarization. The causal relationships between pairs of parameters – $\Delta\psi_p$ and $[Ca^{2+}]_c$, $\Delta\psi_p$ and NAD(P)H, matrix ATP and $[Ca^{2+}]_c$, and $\Delta\psi_m$ and $[Ca^{2+}]_c$ – were investigated at single cell level. It is concluded that, in these β-cells, depolarizing oscillations in $\Delta\psi_p$ are not initiated by mitochondrial bioenergetic changes. Instead, regardless of substrate, it appears that the mitochondria may simply be required to exceed a critical bioenergetic threshold to allow release of insulin. Once this threshold is exceeded an autonomous $\Delta\psi_p$ oscillatory mechanism is initiated.

doi:10.1016/j.bbabio.2012.06.022

P12

Reversibly modulating cytochrome *c* oxidase inhibits blood clotting: Studies based on functional electrochemical models and respiring cells

James P. Collman[1], Chrisopher Barile[1], Lei Fu[2],
Paul Herrmann[3], David Tyvoll[1]
[1]*Stanford University, USA*
[2]*Shanghai Jiao Tong University, China*
[3]*Loma Linda Medical School, USA*
E-mail address: jpc@stanford.edu

Hydrogen sulfide and chelating heterocycles bind reversibly to the Fe and Cu centers in a biomimetic model of cytochrome *c* oxidase (C*c*O), thereby inhibiting the 4-electron reduction of oxygen to water. The same heterocycles have been found to inhibit respiration reversibly in isolated mitochondria. Platelets are important mediators of blood coagulation that lack nuclei, but contain mitochondria. We hypothesized that the inhibition of platelet mitochondria disrupts platelet function and platelet-activated blood coagulation. Indeed, we found that the strength of mitochondrial inhibition correlates with the heterocycle's ability to deter platelet stimulation and platelet-activated blood clotting. These results suggest that for this class of molecules, inhibition of blood coagulation may be occurring through a mechanism involving mitochondrial inhibition.

doi:10.1016/j.bbabio.2012.06.023

P13

Role of conformational change and lipidic ligands in the function and regulation of cytochrome *c* oxidase

Shelagh Ferguson-Miller, Carrie Hiser, Jian Liu, Leann Buhrow
Biochemistry and Molecular Biology, Michigan State University, E. Lansing, MI 48824, USA
E-mail address: fergus20@mail.msu.edu

High resolution crystal structures of bacterial and mammalian cytochrome *c* oxidases (C*c*O) reveal conserved lipid and steroid binding sites [1] as well as redox-linked conformational changes [2], suggesting regulatory and gating possibilities. A bile salt, cholate, is resolved in all crystal structures of bovine C*c*O and forms a tight hydrogen bond with the carboxyl of E62, homologous to E101 in the *Rhodobacter sphaeroides* C*c*O (*Rs*C*c*O), a conserved residue important in K-path proton uptake. These positioning and kinetic studies indicate that the strong inhibitory effect of cholate in bovine C*c*O is due to blockage of the K-path.

Unexpectedly we discovered that μM levels of cholate or deoxycholate stimulate 10-fold the activity of the *Rs*C*c*O mutant, E101A, suggesting chemical rescue of the missing K-path carboxyl. Crystals of *Rs*C*c*O grown in the presence of deoxycholate show a single molecule bound close to E101, in a similar location as cholate in the bovine C*c*O. The conservation of a steroid binding site in such a key position argues for physiological significance. Further studies reveal that a limited set of amphiphilic molecules compete at the entrance of the K-path causing loss or regain of C*c*O activity at μM concentrations.

Also affecting the K-path region is an observed conformational change in the reduced crystals of *Rs*C*c*O [3], causing the loss of steroid binding and the opening of a connection between the K path and the active site. The formation of a new water chain leading into the a_3-Cu_B site indicates a role in gating of proton access. Additional changes observed in the region of heme *a* are similar to some documented by Yoshikawa and coworkers in the reduced bovine C*c*O. The precise physiological and mechanistic significance of the steroid/amphiphile binding site and redox-induced conformational changes remains to be established, but the findings point to new avenues for exploring the regulation of cytochrome *c* oxidase. (Supported by NIH GM26916 to SFM.)

References

[1] L. Qin, C. Hiser, A. Mulichak, R.M. Garavito, S. Ferguson-Miller, Identification of conserved lipid/detergent-binding sites in a high-resolution structure of the membrane protein cytochrome c oxidase, Proc Natl Acad Sci U S A 103 (2006) 16117–16122.
[2] L. Qin, J. Liu, D.A. Mills, D.A. Proshlyakov, C. Hiser, S. Ferguson-Miller, Redox-dependent conformational changes in cytochrome c oxidase suggest a gating mechanism for proton uptake, Biochemistry 48 (2009) 5121–5130.

doi:10.1016/j.bbabio.2012.06.024

P14

ROS production and turnover of brain mitochondria — Implications for neurological diseases

Wolfram S. Kunz
Department of Epileptology and Life & Brain Center, University Bonn, Sigmund-Freud-Str. 25, D-53105 Bonn, Germany
E-mail address: Wolfram.Kunz@ukb.uni-bonn.de

The main superoxide producing sites of brain mitochondria are the FMN of respiratory chain complex I [1] and the center 'o' of bc_1 complex [2]. The complex I formed superoxide is delivered to the mitochondrial matrix, metabolized by MnSOD to H_2O_2 and highly relevant in severe pathology of the brain. The highest rates of superoxide production from this site are detected under the conditions of a highly reduced NAD-system. In contrast, bc_1 complex formed superoxide is predominantly liberated into the intermembrane space, metabolized by Cu,ZnSOD to H_2O_2 and suggested to fulfill signaling functions. The highest rates of superoxide production from that site are detected at intermediate states of coenzyme Q pool reduction in presence of antimycin.

Brain mitochondria are not only major producers of H_2O_2, but they also considerably contribute to the removal of toxic hydrogen peroxide by the glutathione (GSH) and thioredoxin-2 (Trx2) antioxidant systems. By using the specific inhibitors auranofin and DNCB, the contribution of Trx2- and GSH-systems to ROS detoxification in rat brain mitochondria was determined to be $60 \pm 20\%$ and $20 \pm 15\%$, respectively, while catalase contributed to a non-significant extent only. In digitonin-treated hippocampal homogenates inhibition of Trx2- and GSH-systems affected mitochondrial hydrogen peroxide production rates to a much higher extent than the endogenous

extramitochondrial hydrogen peroxide production, pointing to a strong compartmentation of ROS metabolism.

Direct links between mitochondrial ROS production and brain pathology can be observed in brain tissue samples of patients with epilepsy and hippocampal sclerosis. In these samples mitochondrial pathology is related to the accumulation of specific deletions of mitochondrial DNA, which have the 3′ breakpoint at a hot spot at the end of the D-loop region. This localization of the deletion breakpoint implies mitochondrial DNA double strand breaks as mechanism of deletion formation, strongly pointing to ROS as potential mutagenic cause. Thus, ROS-caused formation of mitochondrial DNA deletions, presumably in the vicinity of the FMN site of respiratory chain complex I, is suggested to initiate the epileptogenic process leading to chronic epilepsy.

References

[1] A.P. Kudin, N.Y. Bimpong-Buta, S. Vielhaber, C.R. Elger, W.S. Kunz, J. Biol. Chem. 279 (2004) 4127–4135.
[2] D. Malinska, B. Kulawiak, A.P. Kudin, R. Kovacs, C. Huchzermeyer, O. Kann, A. Szewczyk, W.S. Kunz, Biochim. Biophys. Acta 1797 (2010) 1163–1170.

doi:10.1016/j.bbabio.2012.06.025

P15

Experimental relocation of the mitochondrial *ATP9* gene to the nucleus reveals forces underlying mitochondrial genome evolution

A. Martos[1,2,§], M. Bietenhader[1,2,§], E. Tetaud[1,2,§], R.S. Aiyar[3,§], C.H. Sellem[4], R. Kucharczyk[1,2], S. Clauder-Münster[3], M.-F. Giraud[1,2], F. Godard[1,2], B. Salin[1,2], I. Sagot[1,2], J. Gagneur[3], M. Déquard-Chablat[5,6], V. Contamine[5,6], S. Hermann-Le Denmat[5,6,7], A. Sainsard-Chanet[4], L.M. Steinmetz[3,*], J.-P. di Rago[1,2,*]
[1]*Univ. Bordeaux, IBGC, UMR5095 CNRS, F-33000 Bordeaux, France*
[2]*CNRS, IBGC, UMR5095 CNRS, F-33000 Bordeaux, France*
[3]*Genome Biology Unit, European Molecular Biology Laboratory (EMBL), Meyerhofstrasse 1, 69117 Heidelberg, Germany*
[4]*Univ. Paris-Sud, Centre de Génétique Moléculaire, UPR3404, CNRS, Gif-sur-Yvette F-91198, France*
[5]*Univ. Paris-Sud, Institut de Génétique et Microbiologie, UMR 8621, ORSAY, F-91405, France*
[6]*CNRS, ORSAY, F-91405, France*
[7]*Ecole Normale Supérieure, 75005 Paris, France*
E-mail addresses: larsms@embl.de; JP.dirago@ibgc.u-bordeaux2.fr
[§]These authors contributed equally.

The mitochondrion derives from an ancient alpha-proteobacterium. While the vast majority of its genes have been transferred to the nuclear genome during evolution, a handful of genes remains in all mitochondrial genomes for unclear reasons. Several hypotheses have been proposed to account for the retention of DNA in mitochondria: (i) gene transfer from mtDNA is still underway; (ii) some genes have been confined to the organelle because expressing them from the nucleus would be problematic; and (iii) some genes are retained in order to optimize mitochondrial function. To investigate the evolutionary implications of gene transfer and evaluate potential treatments for the severe diseases caused by mitochondrial mutations, researchers have been attempting to express these genes from the nucleus for decades, however with very limited success. Here we successfully relocate the mitochondrial gene *ATP9* to the nucleus for the first time in any organism, by utilizing naturally nuclear versions of this gene from *Podospora anserina* in *Saccharomyces cerevisiae*. *ATP9* encodes an extremely hydrophobic ATP synthase subunit (9/c). Its genomic locations across species vary considerably, especially in fungi where it can be found in, mitochondrial, nuclear, or even both genomes. We show that reducing the hydrophobicity of subunit 9 is required for crossing the inner mitochondrial membrane when synthesized in the cytosol. Isolation of suppressor mutations that improve allotopic expression of subunit 9 reveals that many additional adaptations beyond the subunit 9 structure are required to optimize its nuclear expression. Our study indicates there must be a compelling reason for *ATP9* to become nuclear, such as the requirement for more specialized regulation of ATP synthase activity by complex, multicellular organisms. This requirement is evident in *P. anserina*, where subunit 9 is expressed from two nuclear genes that are differentially regulated during the life cycle of this filamentous fungus. If there is no benefit for the organism to relocate *ATP9*, this gene would likely remain in the organelle, as in all unicellular species like *S. cerevisiae*, where ATP synthase expression is not subject to any specialized regulation beyond the general glucose-induced repression of respiratory functions. Accordingly, we would like to introduce the hypothesis that variations in the gene content of mitochondria are influenced not only by protein structure, but also by the lifestyle of the organism.

doi:10.1016/j.bbabio.2012.06.026

P16

Dynamic imaging of mitochondrial function, in vivo

Robert S. Balaban
Laboratory of Cardiac Energetics, National Heart Lung and Blood Institute, USA
E-mail address: balabanr@nhlbi.nih.gov

The technology to image mitochondrial dynamics, in vivo, has emerged. These approaches can collect motion corrected micron resolution images over time in viable animals. We use 2-photon excitation schemes to reduce photodynamic damage, improve imaging depth and optimize signal-to-noise ratio (SNR). The improved SNR is realized using total emission detection schemes. A real time 3D motion tracking microscope maintains a field of view on the micron scale for extended periods of time overcoming the major limitation of in vivo imaging, motion. Initial studies on mouse skeletal muscle determined the mitochondria (mito) subcellular distribution and relationship to the vascular system. Surprisingly, capillaries were embedded in the slow twitch fibers (STF) within specialized structures laterally surrounded by large pools of “para-vascular” mito. This pool of mito makes up a significant fraction of the cellular oxidative capacity. These structures enhance and restrict diffusion to slow twitch fibers (STF), which provide a membrane domain for vascular signaling, while localization of mito around the vessels suggests an oxygen sink at the vascular entry site. We monitored the mito NADH fluorescence during a hypoxia, in vivo, to evaluate the metabolic dynamic response as well as the relative reduction levels of cellular mito pools. The para-vascular mito pool was more oxidized than mito in the cell center consistent, but not proving, the possibility of an oxygen gradient due to para-vascular mito oxygen extraction under resting conditions. Mito fission/fusion/motion events are d to play a major role in cellular events. To study this process we constructed a trans-genetic mouse model with a photo-convertible (green to red) EOS protein targeted to mito to follow mito displacements over time. Labeling was obtained in most organs and photo-conversion capabilities were retained. As a control, motion events were observed in cultured fibroblasts from these animals. However, applying this approach to skeletal muscle and skin cells, in vivo, revealed no motion/fusion events for over 45 min. These data