quantified by surface plasmon resonance. NDPK-D was also able to cross-link anionic phospholipid-containing liposomes as seen in light scattering assays, suggesting that the hexameric kinase could promote intermembrane contacts. Mutation of the central arginine (R90) in a surface exposed basic RRK motif unique to NDPK-D strongly reduced these membrane interactions. In a model using HeLa cells naturally almost devoid of NDPK-D, wt protein and R90D mutant were stably expressed, but only wt protein was found attached to membranes. Respiration was significantly stimulated by the NDPK substrate TDP only in mitochondria containing wt NDPK-D, but not in those expressing R90D mutant that is catalytically equally active. This indicates local ADP regeneration in the mitochondrial intermembrane space and a tight functional coupling of NDPK-D with oxidative phosphorylation that depends on the membrane-bound state of the kinase. A model is proposed for a mitochondrial NDPK microcompartment.

39-Plat

Using Two-photon Excited Fluorescence Intensity- and Lifetime-based NADH Imaging to Investigate Cochlea Metabolism

LeAnn M. Tiede, Jorge A. Vergen, Clifford Hecht, Richard Hallworth, Michael G. Nichols.
Creighton University, Omaha, NE, USA.

Metabolism and mitochondrial dysfunction are thought to be involved in many different hearing disorders including noise induced hearing loss and presbycusis. We have employed two-photon fluorescence imaging of intrinsic mitochondrial reduced nicotinamide adenine dinucleotide (NADH) to study the metabolic status of the different cell types in excised yet intact mouse organ of Corti preparations. Recent published studies employ fluorescence lifetime imaging (FLIM) to determine the ratio of the free to enzyme-bound fluorophores populations that occur during changes in metabolism. We have compared traditional intensity based methods to FLIM in order to evaluate the two different methods in both cultured cells and the excised organ of Corti. Treatment with both metabolic uncouplers and inhibitors caused systematic shifts in both the lifetime and populations of the free and bound pools of NADH, resulting in significant differences in the calculated concentration of NADH when compared to using intensity alone to calculate the same value. Mapping of the locations of the individual lifetimes, shows that the lifetime of NADH varies in different cellular locations as well as in different cell types. Possible implications for the study of hearing loss will be discussed.

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40-Plat

Mitochondrial Energy Metabolism and Ca^{2+} Handling in Pancreatic Beta-cells. A System Analysis Approach

Leonid E. Fridlyand, Louis H. Philipson.
University of Chicago, Chicago, IL, USA.

Pancreatic islet beta-cells respond to rising blood glucose by increasing oxidative metabolism, leading to both an increased mitochondrial membrane potential (Ψm) and ATP/ADP ratio in cytoplasm. This leads to a closure of K_{ATP} channels, depolarization of the plasma membrane, influx of calcium and the eventual secretion of insulin. Such a signaling mechanism suggests that mitochondrial metabolism and ATP/ADP ratio regulation in beta-cells may be specially coupled in comparison with other cell types. We performed mathematical modeling to quantitatively assess how cytoplasmic ATP/ADP ratio can be controlled by mitochondria. The cytoplasmic part of the model includes glucokinase, glycolysis, pyruvate reduction, NADH and ATP production and consumption. The mitochondrial part of the model includes production of NADH, which is regulated by pyruvate dehydrogenase. NADH is used in the electron transport chain to establish a proton motive force, driving the F_1F_0-ATPase. Mitochondrial matrix Ca^{2+} is determined by the Ca^{2+} uniporter and Na^+/Ca^{2+} exchanger. The model is described by ordinary differential equations for cytoplasmic and mitochondrial parameters. The model simulates the response of the ATP/ADP ratio to changes in substrate delivery, to inhibition of the mitochondrial Ca^{2+} exchanger and other effects. We found that mitochondrial Ψm should be in a range lower than 150 mV (where F_1F_0-ATPase is sensitive to Ψm) to provide a sensitivity of the ATP/ADP ratio to glucose in beta-cells. On other hand, Ψm can work in the range above 150 mV to provide a maximal F_1F_0-ATPase productivity in other cell types (for example in myocytes). Kinetic analysis of the model reveals that these differences can be simulated by a decreased respiratory activity and higher leak capacity in beta-cell mitochondria in comparison with muscle cell mitochondria that were found recently by Affourtit and Brand (2006).

41-Plat

Modeling Regulation of Mitochondrial Free Ca^{2+} by ATP/ADP-Dependent Ca^{2+} Buffering

Ranjan K. Dash, Matthew D. Thompson, Kalyan C. Vinnakota, Johan Haumann, Mohammed Aldakkak, Amadou K.S. Camara, David F. Stowe, Beard A. Daniel.
Medical College of Wisconson, Milwaukee, WI, USA.

Introduction: Mitochondrial free $[Ca^{2+}]$ ($[Ca^{2+}]_m$) is regulated by cation fluxes through the Ca^{2+} uniporter (CU), Na^+/Ca^{2+} exchanger (NCE), Na^+/H^+ exchanger (NHE), and Ca^{2+}/H^+ exchanger (CHE) as well as via Ca^{2+} buffering by the mitochondrial proteins. However, the regulation of $[Ca^{2+}]_m$ via ATP/ADP-dependent dynamic Ca^{2+} buffering mechanism inside the mitochondrial matrix during transient state-3 respiration is not well known. **Methods:** To gain a quantitative understanding of this Ca^{2+} buffering phenomenon, we developed a computational model of mitochondrial bioenergetics and Ca^{2+} handling by integrating our recent biophysical models of the CU, NCE, NHE, and CHE into our well-validated model of mitochondrial oxidative phosphorylation, TCA cycle, and electrophysiology. The model also accounts for binding and buffering of cations with metabolites, including ATP, ADP and Pi. Experiments were performed to spectrofluorometrically measure $[Ca^{2+}]_m$, pH_m, membrane potential ($\Delta\Psi_m$), and NADH redox state in guinea pig heart mitochondria suspended in Na^+ and Ca^{2+} free buffer medium (ensured with ~50 μM of EGTA) with 0.5 mM pyruvic acid (HPyr). Dynamics were inferred with various addition of $CaCl_2$ (0, 10, 25 μM of $CaCl_2$; 16, 88, 130 nM of free $[Ca^{2+}]$ followed by 250 μM of ADP in the presence or absence of carboxyatractyloside (ANT blocker) and oligomycin (F_1F_0-ATPase blocker). **Results and Discussion:** Model analysis of the data on (i) initial decrease of $[Ca^{2+}]_m$ with addition of Na^+-independent substrate HPyr, and (ii) transient increases of $[Ca^{2+}]_m$ with addition of ADP suggests ATP/ADP-dependent dynamic Ca^{2+} buffering inside the cardiac mitochondrial matrix. This model will be helpful to understand mechanisms by which $[Ca^{2+}]_m$ both regulates, and is modulated by, mitochondrial energy metabolism.

42-Plat

The External Stalk of the FoF1-ATPase: 3D-Structure of the b-Dimer

Oleg A. Volkov, Susan J. Pandey, John G. Wise, **Pia D. Vogel**.
Southern Methodist University, Dallas, TX, USA.

The structure of the external stalk of the FoF1-ATP synthase and its function during catalysis remain one of the important questions in bioenergetics.

Proteomics, structure prediction, molecular modeling and ESR spectroscopy using site-directed spin labeling were employed to elucidate the structure and interfacial packing of the E. coli b-subunit homodimeric and Synechocystis bb' heterodimeric stalks of ATP synthases.

b-Subunits of different origin demonstrate little sequence similarity. Structure prediction algorithms, however, showed that all of the compared sequences contain extensive heptad repeats, suggesting that these proteins may favorably pack as left-handed coiled coils (LHCC).

Molecular modeling of homo- and heterodimeric b produced low energy LHCC. Extensive mutagenesis followed by site-directed spin labeling and subsequent ESR investigations in soluble homo- and heterodimeric b-constructs allowed the determination of inter- and intra-subunit distances.

Inter-spin distances obtained by ESR agreed very well with distances derived from LHCC molecular models of b- and bb'-dimers and therefore strongly support our proposition that dimeric external stalks of ATP synthases indeed form left-handed coiled coils.

The extreme C-terminal part of the b-dimer is not predicted to form a coiled coil structure. We are presently investigating this part of the second stalk both when in solution and when in complex with soluble F1-ATPase. The influence of subunit δ is of particular interest due to its proposed direct interaction with the C-terminus of subunit b.

Initial site-directed spin labeling and ESR experiments using complete E. coli FoF1-ATP synthase indicate that the inter-subunit packing of the b-dimer changes during catalytic turnover, which may be a mechanism for elastic coupling of the different rotating parts of the enzyme.

43-Plat

Torque Generation Mechanism of ATP Synthase and Other Rotary Motors

John H. Miller[1], Vijayanand Vajrala[1], Hans L. Infante[1], James R. Claycomb[2].
[1]University of Houston, Houston, TX, USA, [2]Houston Baptist University, Houston, TX, USA.

Ion driven rotary motors, including F0-ATP synthase (F0) and the bacterial flagellar motor, convert energy from ion translocation into torque and rotary