1259-Pos Board B103

Characterization of a UCP-like Activity in *Yarrowia lipolytica*

Luévano-Martínez Luis Alberto[1], Moyano Eva[2], Rial Eduardo[2], Uribe-Carvajal Salvador[1].

[1]Instituto de Fisiología Celular, UNAM, Mexico City, Mexico, [2]Centro de Investigaciones Biolígicas, CSIC, Madrid, Spain.

Uncoupling proteins (UCP's) belong to the mitochondrial six-transmembrane segment carriers. Among the five known isoforms only UCP1 (thermogenin) is well characterized physiologically. In addition to shivering thermogenesis UCP1 produces heat hence aiding to maintain an optimum body temperature. The function of the other isoforms is not so well understood, since they are expressed in non-thermogenic tissues such as skeletal muscle, brain, kidney, etc. However, it is has been seen that they are over-expressed in several stress conditions, probably with the aim to reduce the production of reactive oxygen species (ROS). In plants, UCP's, are used to evaporate allelopathic compounds and pheromones through an increase in tissue temperature. In recent years several UCP's have been discovered in unicellular organisms such as Candida parapsilosis, Aspergillus fumigatus and Acanthamoeba castellanii, indicating that UCPs are distributed in all eukaryotic kingdoms. Recently we have observed an UCP-like activity in Yarrowia lipolytica's mitochondria, i.e. these mitochondria are uncoupled by fatty acids and this effect is inhibited by purine nucleotides (ATP, ADP or GDP). The same antagonist effect is observed at the level of the mitochondrial membrane potential ($\Delta\psi$), that is $\Delta\psi$ is collapsed by fatty acids and increased by purine nucleotides. We have searched for a possible UCP's gene the genome of this yeast and have found two candidates. The first one is annotated as an oxodicarboxylate carrier and the second one as an oxaloacetate carrier. To corroborate whether one of these genes codes for a UCP, we expressed these proteins in Saccharomyces cerevisiae and sought for new UCP-like sensitivity to fatty acids and to purine nucleotides.

1260-Pos Board B104

Dysfunctional Mitochondria In Hearts From Type 1 Ryanodine Receptor (RyR1) Knockout Mice

Gisela Beutner, Robert T. Dirksen, **Shey-Shing Sheu**.

University of Rochester, Rochester, NY, USA.

Mutations in the genome of the cardiac or skeletal ryanodine receptor subtypes (RyR2 and RyR1, respectively) cause diseases like catecholaminergic polymorphic ventricular tachycardia, malignant hyperthermia, or central core disease. The objective of this study is to investigate the consequences of a dysfunctional ryanodine receptor type 1 (RyR1) on the functionality of heart mitochondria.

In neonatal control mice, cardiac mitochondrial oxygen consumption was 20.68 $\pm$ 2.11 nmol/min/mg protein in the presence of 2 mM succinate or 5 mM malate and 5 mM glutamate. Addition of 1 mM ADP, to induce state 3 respiration, increased oxygen consumption significantly to 52.37 $\pm$ 2.14 nmol/min/mg (n=5). On the contrary, cardiac mitochondria from RyR1 knockout mice showed no increase of oxygen consumption upon addition of 1 mM ADP or 10 μM dinitrophenol. The respiratory control index of RyR1 knockout mice was 1.16 $\pm$ 0.48 (n=4) compared to 2.42 $\pm$ 0.38 in control mice (n=5).

Atractyloside, a specific inhibitor of the adenine nucleotide translocase (ANT) did not inhibit oxygen consumption in cardiac tissue homogenates from RyR1 knockout mice. Consistent with this finding is that ANT protein was not detected using specific antibodies in heart mitochondria from RyR1 knockout mice (n=45), while VDAC and cytochrome c protein levels were not different. Lastly, expression mitochondrial creatine kinase was higher in RyR1 knockout mice than in control animals (n=8). However, mitochondrial contact sites, consisting of VDAC, the creatine kinase octamer and ANT, were absent in heart mitochondria from RyR1 knockout mice.

These results suggest that RyR1 knockout mice have dysfunctional mitochondria and are unable to consume oxygen for ATP production. These findings are consistent with the notion that mitochondrial RyR1 plays a central role in Ca^{2+} regulated ATP production in the heart.

Supported by HL-33333.

1261-Pos Board B105

Probing membrane proteins: Monitoring proton-translocation quantitatively

Thom Leiding[1], Sergei Vinogradov[2], Cecilia Hägerhäll[1], Sindra Peterson Årsköld[1].

[1]Center for Chemistry and Chemical Engineering, Lund University, Lund, Sweden, [2]Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA, USA.

Proton-translocating membrane-bound proteins create and maintain pH gradients across membranes, playing important bioenergetic and regulatory roles. Some of these proteins are implied in ageing and neurodegeneration, others in tumor biology and drug resistance. Unfortunately, membrane proteins are notoriously difficult to study. They are vastly underrepresented in the growing body of protein structures, and recent proteomics advances have mostly involved soluble proteins.

We present a system for quantitatively monitoring proton-translocation by membrane proteins. The protein is reconstituted into large vesicles, which provide a well defined environment. Optionally, the protein can be first fused to a cytochrome, facilitating unidirectional reconstitution[1]. The pH inside and outside the vesicles is monitored simultaneously using novel membrane-impermeable nanoprobes[2,3]. Using CCD detection, full spectral coverage of the nanoprobes and any protein-bound chromophores is delivered at up to ms time resolution. The proteoliposomes are then subjected to computer-controlled, semi-automated titrations of substrate and/or pH changes[4].

Key improvements over similar methodologies previously employed are the advantageous characteristics of the nanoprobes, the precision and speed of the titration system, and the CCD detection technology. Combined, these features ensure robust, fast, quantitative results on proton-pumping stoichiometries across membranes.

Using this method, we characterize the lipid bilayer of the vesicles in terms of passive ion leakage and stability. We then investigate subunits of respiratory Complex I, and their antiporter homologues, reconstituted into liposomes (see Sindra Peterson Årsköld's poster).

1. Gustavsson, T., Eek, M., Leiding, T., Peterson Årsköld, S., and Hägerhäll, C. (2008) Submitted to Biochimica et Biophysica Acta Bioenergetics.

2. Finikova, O., Galkin, A., Rozhkov, V., Cordero, M., Hagerhall, C., and Vinogradov, S. (2003) J. Am. Chem. Soc. 125, 4882-4893.

3. Leiding, T., Górecki, K., Vinogradov, S., Hägerhäll, C., and Peterson Årsköld, S. (2008) Submitted to Analytical Biochemistry.

4. Autonomous Science Machines TM.

1262-Pos Board B106

Cell-Free Electrophysiology of Native Inner Mitochondrial Membranes

Petr Obrdlik, Natalie Watzke, Kerstin Diekert, **Bela Kelety**.

IonGate Biosciences, Frankfurt Main, Germany.

Transporters, pumps and channels of inner mitochondrial membranes are involved in all central mitochondrial functions such as regulation of ATP synthesis, thermogenesis, control of cellular calcium, regulation of apoptosis, and in balancing production of cellular reactive oxygen species. However, functional investigation of mitochondrial transport proteins is tedious. Typically, the functional studies rely on the reconstitution of recombinant proteins in proteoliposomes. This work describes the use of the SURFE2R technology for electrical measurements of transporters in native mitochondrial membranes. The inner mitochondrial membranes were isolated from pig heart and adsorbed on gold electrodes according to standard protocols. These biosensors were measured with the standard SURFE2R equipment. Three transport proteins were characterized: the ADP/ATP exchanger (ANT), the F-type ATPase (complex V), and the Cytochrome c Oxidase (COX, complex IV). The transport was activated through substrate concentration jumps (ATP or ADP in case of ANT and F-type ATPase) or, in case of COX, via concentration jumps with reduced cytochrome c. In case of activation via ATP-concentration jumps, both, the ANT and F-type ATPase activity could be detected independently on the same sensor by applying ANT- or ATPase-selective solutions. The specificity of the currents was verified by ATPase- and ANT-specific inhibitors. As expected, the ATP-induced activity of ANT was increased in presence of ADP gradients across the membranes. The ANT-signals depended on the applied ATP-concentration. The cytochrome c-induced currents were inhibited by COX-specific inhibitor cyanide and depended on the redox state of the protein. In summary, the presented results demonstrate a novel, easy and reliable approach for functional studies of mitochondrial transporters and pumps in their native surroundings, and can potentially help to understand the complex molecular mechanisms of different mitochondrial functions. Additionally, the technology platform can also be used to perform pharmacological screens for mitochondria-targeted drugs.