an inactive mutant enzyme for earlier studies but recently succeeded to reveal the structure of the wild type enzyme. We are going to update the current status of the study using this and earlier structures of LacY in comparison with other transporter structures. Eukaryotic membrane proteins are often difficult to produce in large quantities, which is a significant obstacle for further structural and biochemical investigation. Recently, we have reported a fluorescent-based high-throughput approach for rapidly screening membrane proteins that can be overproduced to levels of >1 mg/l in *Saccharomyces cerevisiae*. We find that 70% of the well-expressed membrane proteins tested in this system are stable, targeted to the correct organelle, and monodispersed. In the workshop, we will present the results of the application of this method to the production of various mammalian transporters, which are successfully purified in large quantity. We could also show that the system can, in fact, produce active mammalian transporters. We will discuss the application of this system to functional and structural studies of mammalian transporters.

doi:10.1016/j.bbabio.2008.05.101

S3/3 Crystal structure based study of NhaA, a Na^+/H^+ antiporter

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The Na^+/H^+ antiporter NhaA is indispensable for pH and Na^+ homeostasis in *Escherichia coli* and many other bacteria. It has unique properties; in addition to being a transporter it has a capacity to sense the environmental signals, Na^+ and H^+ and to transduce the signals into a change in activity so as to maintain homeostasis. Whereas, the response to Na^+ occurs at the transcription level, the response to H^+ is conducted by the protein itself. Similar to many prokaryotic and eukaryotic antiporters NhaA is tightly regulated by pH. The crystal structure of NhaA has provided insights into the mechanism of NhaA and its unique regulation by pH. Being a novel fold, it has also shed light on the architecture of membrane transport proteins and provided a basis to intelligently design experiments both in-silico and in the molecule to study the mechanism of an antiporter and its regulation. The aim of this lecture is to describe this enlightening encounter between the crystal structure and the molecular membrane biology of NhaA.

doi:10.1016/j.bbabio.2008.05.102

S3/4 Substrate recognition and transport mechanism of mitochondrial carriers

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Mitochondrial carriers transport nucleotides, co-factors and metabolic intermediates across the mitochondrial inner membrane. The significant sequence conservation in the mitochondrial carrier family suggests that the specific recognition of substrates is coupled to a common mechanism of transport. By using two different approaches a common substrate binding site was identified consisting of residues that are highly conserved and essential for function. The first approach uses comparative structural models and chemical and distance constraints to identify a substrate binding site capable of discriminating different substrates. The second exploits the principle that mitochondrial carriers have a high degree of three-fold pseudosymmetry in contrast to the transported substrates that are asymmetric in structure. Therefore, the substrate binding site must contain asymmetric and conserved residues to couple the binding of the asymmetric substrate to a symmetric transport mechanism. A symmetry score based on sequence comparisons was devised to asses the degree of symmetry and conservation in the carriers. Conserved asymmetry residues are found predominantly in the cavity at the midpoint of the membrane in agreement with the first approach. The common substrate binding site explains substrate selectivity, ion coupling and the effects of the membrane potential on transport. In addition, the symmetry analysis has identified residues that are important for the transport mechanism of mitochondrial carriers.

doi:10.1016/j.bbabio.2008.05.103

S3/5 Control and effect of UCP1 activity in brown-fat cells and mitochondria, and in mice and men

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Regulation of UCP1 activity has both biochemical and applied interest. However, the results are dependent upon the level of biological integration used to examine UCP1 activity.

Thus, in the simplest systems – black-lipid membranes and reconstituted vesicles – fatty-acids activate dependent upon e.g. degree of unsaturation, they have to be flipflopable and ROS products seem to affect activity. When UCP1 is studied in brown-adipose-tissue mitochondria, the results are different: fatty-acids reactivate GDP-inhibited UCP1 but in an unsaturation-independent manner, they need not be flipflopable and ROS products do not affect. When hyperactivation of uninhibited UCP1 is studied, similar results are obtained. When UCP1 is studied within brown-fat cells, it can be (re)activated indirectly by norepinephrine or directly by fatty-acids. The fatty-acids need not be metabolizable, at least not beyond the acyl-CoA level. When UCP1 activity is studied within intact animals (mice), the outcome depends on temperature conditions: at normal animal house conditions, nearly no effects are seen; in the cold, nonshivering thermogenesis cannot be induced, and at thermoneutrality, UCP1 may control body weight, i.e. its absence leads to obesity. Although UCP1 was thought until recently to be absent in adult humans, its presence is now evident, and through FDG-PET its activity can be studied and correlated with physiological conditions.

doi:10.1016/j.bbabio.2008.05.104

S3/6 A novel potassium channels in mitochondria

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Potassium channels (ATP-regulated, calcium activated and voltage dependent potassium channels) present in inner mitochondrial membranes were implicated in cytoprotective phenomenon in various tissues. These channels modulate mitochondrial matrix volume, mitochondrial respiration and membrane potential, and generation of reactive oxygen species. In this paper we describe the biophysical and pharmacological properties of new mitochondrial potassium channels recorded in *Acanthamoeba castellanii* and potato tuber mitochondria. Additionally, properties of mitochondrial potassium channels present in neuronal, cardiac tissue and endothelial cells will be described.

This work was supported by grants from MNiSW P-N/031/2006, N30105331/1676 and Polish Mitochondrial Network MitoNet.pl.

doi:10.1016/j.bbabio.2008.05.105

S3/7 Intramitochondrial signaling — Interactions among mitoK$_{ATP}$, PKCε, ROS, and MPT

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Our aim was to apprehend the pathways by which mitoK$_{ATP}$ opening leads to inhibition of the mitochondrial permeability transition (MPT), thereby reducing ischemia–reperfusion injury. We showed previously that mitoK$_{ATP}$ is opened by activation of a mitochondrial PKCε, designated PKCε1, that is closely associated with mitoK$_{ATP}$. MitoK$_{ATP}$ opening causes an increase in ROS production by Complex I of the respiratory chain. This ROS activates a second pool of PKCε, designated PKCε2, which inhibits the mitochondrial permeability transition (MPT). We measured mitoK$_{ATP}$-dependent changes in mitochondrial matrix volume to further investigate the relationships among PKCε, mitoK$_{ATP}$, ROS, and MPT. We present evidence that (1) H_2O_2 and NO cause mitoK$_{ATP}$ opening that is mediated by PKCε1 and not by direct actions on mitoK$_{ATP}$; (2) superoxide has no effect on mitoK$_{ATP}$ opening; (3) H_2O_2 or NO inhibits MPT opening, and both compounds do so independently of mitoK$_{ATP}$ activity via activation of PKCε2; (4) mitoK$_{ATP}$ opening induced by PKG, PMA or diazoxide is not mediated by ROS; and (5) mitoK$_{ATP}$-generated ROS activates PKCε1 and induces phosphorylation-dependent mitoK$_{ATP}$ opening *in vitro* and *in vivo*. Thus, mitoK$_{ATP}$-dependent mitoK$_{ATP}$ opening constitutes a positive feedback loop capable of maintaining the channel open after the stimulus is no longer present. This feedback pathway may be responsible for the lasting protective effect of preconditioning, colloquially known as the memory effect.

doi:10.1016/j.bbabio.2008.05.106

(S3) Membrane transporters symposium abstracts (poster and raised abstracts)

S3.8 Effects of inhibitors on the unfolding of the mitochondrial ADP/ATP carrier by single-molecule force spectroscopy

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The mitochondrial ADP/ATP carrier exchanges cytosolic ADP for ATP synthesised in the mitochondrial matrix and replenishes the eukaryotic cell with metabolic energy. Two specific inhibitors of the carrier are known; atractyloside (ATR) and carboxyatractyloside (CATR), which differ in one carboxylate. Reconstituted histidine-tagged yeast ADP/ATP carrier AAC3 with either ATR or CATR bound was subjected to single-molecule force spectroscopy. The amino-terminal end of the protein was pulled out of the α-helical bundle in pairs of helices, reflecting the tripartite structure of the carrier. Additional resistance to unfolding was observed on helix H2 when CATR was bound rather than ATR. Two-dimensional NMR spectroscopy was used to confirm the stereochemistry of ATR, showing that the additional carboxylate of CATR is in the equatorial position. We interpret the extra resistance to be caused by the removal of the inhibitor together with the first two α-helices of the carrier, as the inhibitor is bound most strongly to these α-helices. The single-molecule force spectroscopy studies explain why CATR confers additional structural stability to the carrier.

doi:10.1016/j.bbabio.2008.05.107

S3.9 Effect of single gene deletions of *mrpA–G* and *mrpE* point mutations on activity of the Mrp Na^+/H^+ antiporter of alkaliphilic *Bacillus* and formation of hetero-oligomeric Mrp complex

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The putative "multi-subunit" Mrp family of secondary monovalent cation proton antiporters is physiologically important in diverse bacteria. The aim of this study was to examine structure–function of the product of the seven-gene *mrp* operon from an alkaliphilic *Bacillus*. The cloned operon was engineered so that each of the Mrp proteins (MrpA–G) could be detected. When expressed in an antiporter-deficient strain of *Escherichia coli*, Mrp-dependent $Na^+(Li^+)/H^+$ antiport was observed. Analyses by combined Blue Native electrophoresis and SDS-PAGE demonstrated complexes that contain all 7 gene products in size ranges that could be monomers and dimers. Analyses of single, nonpolar *mrp* gene deletion mutants showed that: all Mrp proteins were required for significant antiport activity; MrpD is required for stable membrane incorporation of all other Mrp proteins;