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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