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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.

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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.

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(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.

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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;

and a catalytically inactive MrpA–D sub-complex is formed in the absence of MrpE, F or G. Studies of *mrpE* point mutations further demonstrated the strong influence of small changes in MrpE on the antiport properties of the whole complex although MrpA and D have been hypothesized to be the actual antiporter subunits.

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S3.10 A role for uncoupling protein 1 in the formation of the mitochondrial permeability transition pore?

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The mitochondrial permeability transition pore (mPTP) is a non-specific channel that forms in the inner mitochondrial membrane in response to elevated levels of matrix calcium. The pore is generally believed to be comprised of the adenine nucleotide translocase (ANT), as well as several other mitochondrial proteins (e.g. VDAC and Cyclophilin D). Recent studies, however, indicate that the presence of ANT is not essential for permeability transition, which has led to the proposal that other members of the mitochondrial carrier protein family may be able to play a similar function to ANT in pore formation. To investigate this possibility we are studying the permeability transition properties of brown adipose tissue (BAT) mitochondria in which levels of the mitochondrial carrier protein, UCP1, can approach those of ANT. Using BAT mitochondria isolated from both wild-type and $UCP1^{-/-}$ mice we assess UCP1-specific, membrane potential-independent, influences on mPTP formation by studying their swelling properties under strictly de-energised conditions. UCP1-dependent contributions to mPTP formation will, therefore, allow us to determine if the mitochondrial carrier protein involvement in permeability transition is a more general property of this family of proteins or is more likely to be restricted to specific members such as the ANT.

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S3.11 The mechanism of transport across the inner mitochondrial membrane: Clues from the PxW subfamily

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Mitochondrial carrier family members possess three conserved peptides that structural studies show to be linked by ionic bonds integral to opening the transport barrier into the mitochondrial matrix. These peptides contain the Px(D/E)xx(R/K) motif across family members, but a small subset of these carriers substitute a PxWxx(R/K) motif in the second peptide, and hence, possess new information about the mechanism of carrier opening. We have been applying site-directed mutagenesis to investigate the role of this PxWxx(R/K) motif in the mitochondrial folate transporter (MFT). We mutated MFT conserved sequence residue R249 and concluded that a π–cation interaction replaced one of the ionic bonds, which was consistent with previously proposed molecular modeling studies. Current results demonstrate that the MFT evolved a W (W142) substitution in place of the (D/E) in the second peptide because an acidic residue in that position is incompatible with MFT function. Computational studies suggest that the aromatic phenyl ring of the folate substrate likely facilitates breakage of a π–cation interaction. A mutation predicted to form an additional π–cation interaction with W142 and stabilize the transport barrier, eliminated MFT function. Thus, the energetics of breakage of the transport barrier are delicate and tuned to the characteristics of the substrate.

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S3.12 The ADP/ATP carrier of the apicomplexa *Cryptosporidium parvum*

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The eukaryotic parasite *Cryptosporidium parvum* has mitochondria with reduced functions. Consistent with this notion, we have identified only eight putative transport proteins of the mitochondrial carrier family (MCF). Members of the MCF support mitochondrial metabolic energy generation, macromolecular synthesis, and amino-acid metabolism by linking biochemical pathways in the mitochondrial matrix with those in the cytosol. One of the *Cryptosporidium* proteins has been expressed functionally in the bacterium *Lactococcus lactis* and has been identified by whole cell and fused vesicle uptake studies as the ADP/ATP carrier. The transporter has a similar substrate specificity and inhibitor profile as the bovine and yeast ADP/ATP carrier. By comparative modelling, the internal cavity of the ADP/ATP carrier is more akin to the bovine ADP/ATP carrier than that of the ADP/ATP carrier of *Entamoeba histolytica*, which is the organism that causes amoebic dysentery.

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S3.13 Determination of the dimensions and mass of membrane proteins in detergents by size exclusion chromatography

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Determining the oligomeric state of a membrane protein is an important step in understanding its mechanism. Here, we apply a new approach based on size exclusion chromatography (SEC) of membrane proteins in the alkyl-maltoside and Cymal detergent series. The procedures will be illustrated by using the yeast mitochondrial ADP/ATP carrier, which was shown to be monomeric in detergent rather than dimeric. Several parameters of the detergent-solubilised protein were determined, such as its molecular mass, its Stokes' radius, its radius at the midpoint of the membrane, and its excluded volume. The procedures can be used to determine the mass and dimensions of other membrane proteins when the chromatographic behaviour in SEC is determined largely by the associated detergent micelle.

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