aborted cytoplasmic state of the carrier has been determined [4], but no structures are available for the empty or substrate binding states. The aim is to probe the substrate binding and conformational changes of the ADP/ATP carrier by measuring changes in labelling efficiency of single-cysteine residues with membrane-impermeable sulphydryl reagents.

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3P.2 Identification of the mammalian mitochondrial pyruvate carrier

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The existence of a specific mitochondrial carrier for pyruvate was first demonstrated in this laboratory by the use of the specific inhibitors, α-cyano-4-hydroxycinnamate (CHC) and its more potent analogue UK-5099, that reversibly modify a thiol group on the carrier. The yeast mitochondrial pyruvate carrier (MPC) was subsequently identified by measuring inhibitor-sensitive pyruvate uptake, from 18 different *Saccharomyces cerevisiae* mutants, each lacking a member of the mitochondrial carrier family (MCF). Only mitochondria from the YIL006w deletion mutant were transport deficient and this gene encodes a 41.9 kDa member of the mitochondrial solute transporter family [1]. However, others have reported this protein to be the NAD^+ transporter [2]. The nearest mammalian homologues to YIL006w are the mitochondrial folate carrier SLC25A32 (30% identity), SLC25A33 (28% identity) and SLC25A36 (28% identity). We have used two techniques to try and identify which, if any, of these is the mammalian MPC. First we have investigated whether CHC and UK-5099 can protect a specific mitochondrial inner membrane protein from labelling by fluorescent maleimide derivatives. Fluoranthyl maleimide and pyrene maleimide were identified as the most appropriate maleimide derivatives since these were found to inhibit mitochondrial pyruvate transport. However, no consistent protection of a protein was identified on 2D gels although this might reflect the recognised problems associated with separating members of the MCF by this technique. Progress in resolving these problems will be presented. Second, we are using siRNA to knockdown the candidate genes for the MPC in cultured cell lines (SiHa cells). This has required the development of an assay for mitochondrial pyruvate transport in these cells which is a technical challenge because the yield of mitochondria is too small to perform radioactive transport assays. However, we have developed an indirect assay that can detect small changes in MPC activity *in situ* and we will present data that uses this technique to screen for the mammalian MPC.

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3P.3 Determining the oligomeric state of mitochondrial carrier proteins by blue native gel electrophoresis

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Mitochondrial carriers were widely believed to be homo-dimers comprised of two about 32 kDa subunits. In blue native polyacrylamide gel electrophoresis (BN-PAGE) studies mitochondrial carriers from detergent-solubilised mitochondria migrate in the range of 70–120 kDa, which was interpreted to represent homo-dimers. Work from our laboratory has shown that the ADP/ATP carrier has an apparent mass in the range of 67–134 kDa when purified by size exclusion chromatography in the alkyl-maltoside detergent series. However, the carrier is a monomer, but the mass contribution of the associated detergent–lipid micelles varies. The effects of lipids and detergents on the migration of mitochondrial carriers in BN-PAGE have not been determined. Here, BN-PAGE was used to determine the apparent mass of the yeast ADP/ATP carrier AAC3. Our experiments show that AAC3 in dodecyl maltoside migrates at about 130 kDa species when solubilised from mitochondrial membranes, but at about 60 kDa species when purified. Importantly, the difference in apparent mass does not relate to a difference in oligomeric state, as the detergent micelle of the carrier solubilised from mitochondria has higher lipid content than the purified protein. When solubilised from mitochondrial membranes at higher detergent concentrations, the apparent mass of AAC3 decreases to about 70 kDa as the associated lipid is diluted away from the protein by detergent. The stepwise re-introduction of mitochondrial lipid to the purified protein leads to an increase in the apparent mass of AAC3 to about 130 kDa. Importantly, the incremental changes in mass are too small to account for a change in the oligomeric state of AAC3. When purified in various alkyl maltoside detergents, AAC3 decreases in apparent size with decreasing length of the detergent alkyl chain (from about 65 kDa in tri-decyl maltoside to about 45 kDa in decyl maltoside). We conclude that in previous studies the observed masses were incorrectly interpreted in favour of the dimer, as the contribution of lipid and detergent to the migration of the mitochondrial carriers in BN-PAGE had not been fully appreciated.

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3P.4 The new type of uncouplers which selectively interact with non-equilibrium membrane bounded protons

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In the previous work [1] we detected some interesting properties of a derivative of 2,4,6-threechlorine-3-pentadecylphenol (TCP-C15). This compound has very high affinity to membranes as compared to most of classical uncouplers of oxidative phosphorylation. TCP-C15 at concentrations of 10–60 μM was shown to selectively interact with the proton fraction [2,3] interacting with membrane under non-equilibrium conditions, as distinct from classical uncouplers which have more hydrophilic properties. It should be referred to a new class of uncouplers. Meanwhile, the question if the action of this compound was due to the detergent effect (the consequence of

membrane damage) or are we dealing with a new proton transporter was open. In this work, TCP-C15 was shown to slowly incorporate into membrane. Therefore, by long incubation (10–20 min) we showed an authentic uncoupling effect (stimulation of mitochondrial respiration) with nanomolar concentrations of this compound. With the help of a new experimental method, it was shown that the mitotropic compound of SkQ set, which in reductive conditions in the mitochondrion transforms into the hydroquinone form, shows uncoupling effect at nanomolar concentrations.

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3P.5 Establishing the role of the mitochondrial carrier MTCH2

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Mitochondrial outer-membrane permeabilization (MOMP), leading to cytochrome *c* release and caspase protease activation is a major event during apoptosis. BCL-2 family members are pivotal regulators of MOMP, but it is unknown how they regulate this process. BID, a pro-apoptotic BCL-2 family member, plays a critical role in executing apoptosis in the liver by initiating MOMP. Previously, mitochondrial carrier homolog 2 (MTCH2) was demonstrated to interact with BID in cells signaled to die. MTCH2 is a novel and previously uncharacterized 33-kDa protein, which belongs to the mitochondrial carrier family. By using a conditional knockout mouse MTCH2 was demonstrated to be essential for BID-induced apoptosis in the liver, but its mechanism of action remains unknown. A detailed bioinformatic analysis was carried out to assess how closely related MTCH2 is to other mitochondrial carriers. MTCH2 has the same structural features and topology as other mitochondrial carriers, but it has a unique substrate binding site, suggesting that the putative substrates differ chemically and structurally from known carrier substrates. In addition, MTCH2 has incomplete matrix and cytoplasmic salt bridge networks, indicating a low energy barrier to conformational changes. How will the substrates of MTCH2 be identified? The observation that MTCH2 has a unique substrate binding site with well-defined chemical features may help to narrow down substrate candidates. In addition, MTCH2 orthologs do not exist in yeast and plants, indicating that the substrate either does not exist or does not need to be transported into mitochondria in these organisms. To screen for substrate candidates the human MTCH2 was expressed in the cytoplasmic membrane of the Gram-positive bacterium *Lactococcus lactis*. The main advantage of using this system is that transport assays for the identification of the substrate can be performed with whole cells, as the carrier is directly accessible to the provided substrates. Establishing the role of MTCH2 as a mitochondrial carrier may have important implications for mitochondrial metabolism and the regulation of MOMP by BCL-2 family members during apoptosis.

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3P.6 Identification and characterization of functional residues in a multi-subunit type Na^+/H^+ antiporter Mrp complex from alkaliphilic *Bacillus pseudofirmus* OF4

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Mrp antiporters catalyze secondary $Na^+(Li^+)/H^+$ antiport and/or K^+/H^+ antiport that is physiologically important in diverse bacteria. Mrp is unique among antiporters in requiring all six or seven hydrophobic gene products (MrpA to MrpG) of the *mrp* operon for antiport activity. The MrpA, D and C proteins of the MrpA-B-C-D module have sequence similarity with Complex I subunits whereas the MrpE-F-G module is Mrp-specific [1,2]. A panel of site-direct mutants in 28 conserved or specific motif-related amino acid residues from an alkaliphile Mrp Na^+/H^+ antiporter was constructed. Each mutant transporter was expressed in antiporter-deficient *Escherichia coli* strain KNabc and the mutations were classified into 7 groups based on assays of growth/sodium-resistance, antiport properties, Mrp subunit levels, and formation of monomeric and dimeric Mrp complexes that are the active forms [1]. The analyses identified charged residues that are important for antiport activity and that are conserved across the large Mrp subunits of the MrpA-D module, MrpA, MrpD, as well as membrane-bound subunits (NuoL/M/N) of complex I. These included MrpA-K223, -K299 and MrpD-K219 as well as acidic residues that had been identified in *Bacillus subtilis* Mrp [3]. This study also extended evidence for a key role for MrpE of the MrpE-F-G module. MrpE is required for normal membrane levels of other Mrp proteins and normal complex formation. Conversely, some mutations in the MrpA-to-D module affected membrane levels of MrpE. Residues that are required for formation of the monomeric form or both forms of hetero-oligomeric Mrp complexes were identified for the first time. A mutation of Proline81 in MrpG produced a novel Mrp that supported sodium-resistance but lacked antiport activity. While a pair of tyrosines and a VFF motif with proposed roles in sodium-binding were mutated without effect, mutation of MrpA-H700 of a putative quinone binding site affected K_m values for the activity.

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3P.7 Effect of cardiolipin on the iron uptake of F_1F_OATP synthase in heart mitochondria

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Excessive iron is known to amplify ROS by Fenton and Haber–Weiss reaction, with subsequent damage of proteins, lipids and DNA of mitochondria. As one of the components affected by iron, cardiolipin, a tetra-acyl phospholipid, is crucial for oxidative phosphorylation of mitochondria. Recently, we showed that iron uptake was