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