to characterise wild type and mutant proteins by polarographic and voltametric techniques to investigate the importance of these residues in ubiquinone-binding. Q242N, S256T and R262K substitution results in complete loss of AOX electron transfer activity that is not due to instability of the protein suggesting that these residues, also identified as being important in complex II, are critical for Q-binding in AOX. Of equal interest was the finding that N247Q substitution had little effect upon electron transfer or inhibitor sensitivity. We are currently investigating the importance of this residue since we believe it is responsible for a difference in sensitivity to ascofuranone, a very specific inhibitor of the alternative oxidase, in *Trypanosoma brucei* and *Trypanosoma vivax*. Results will also be presented as to the importance of Y253 and H262 in Q-binding.Supported by the BBSRC.

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S11.40 Over-expression, purification and crystallisation of the alternative oxidase

Catherine Elliott[a], Mary S. Albury[a], Momi Iwata[b], Anthony L. Moore[a]
[a]*Biochemistry and Biomedical Sciences, University of Sussex, Brighton, UK*
[b]*Centre for Structural Biology, Imperial College, London, UK*
E-mail: ce49@sussex.ac.uk

The aim of this project is to elucidate the structure of the alternative oxidase under optimal crystallographic conditions. The alternative oxidase (AOX), an inner-mitochondrial membrane ubiquinol:oxygen oxidoreductase, is responsible for cyanide-resistant respiration in plants, several fungi and a variety of alpha-proteobacteria. Importantly, several human parasites, including *Trypanosoma brucei brucei* and *Blastocystis hominis*, also functionally express the alternative oxidase gene. In the case of trypanosomes, the causative agent of African sleeping sickness, AOX has been found to be the sole oxidase present in the bloodstream form of the kinetoplast parasite. While the alternative oxidase has been modeled *in silico*, the exact structure remains unknown. Detailed knowledge of the structure is essential to the future study of the enzyme, specifically in relation to rational drug design of effective anti-parasitic drugs. *Sauromatum guttatum* AOX has been over-expressed in both C41 and heme-deficient *Escherichia coli* strains, solubilised in the presence of a variety of detergents, and subsequently purified using cobalt affinity gel. Results will be presented to show the production of an active protein at all stages of the purification process, in addition to demonstrating for the first time that the plant AOX is sensitive to the specific trypanosomal inhibitor ascofuranone. This work is supported by a grant from the BBSRC.

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(S12) Mitochondria and disease symposium lecture abstracts

S12/1 Mitochondria function in the diabetic kidney

Fredrik Palm
Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden
Department of Medicine, Georgetown University, Washington, DC, USA
E-mail: fredrik.palm@mcb.uu.se

The diabetic kidney has an altered energy metabolism which is partly due to the increased tubular electrolyte load, but also due to activation of several seemingly different pathways. We have previously shown that increased oxidative stress and activation of the polyol pathway result in reduced tissue oxygen tension throughout the diabetic kidney. Interestingly, we found that the reduced oxygen availability always was linked to increased oxygen utilization, even during situations of glomerular hypofiltration, i.e. when the tubular load of electrolytes was reduced.

We could show that the increased oxygen utilization in the diabetic kidney *in vivo* is dependent on reduced nitric oxide bioavailability, and thus reduced inhibition of mitochondria respiration, but exogenous stimulation of the nitric oxide production was not sufficient to alone normalize the oxygen tension. When investigating the cellular oxygen metabolism in the diabetic kidney at the molecular level, we found that the diabetic kidney has increased uncoupling protein (UCP)-2 expression. Oxygen consumption by diabetic mitochondria can be stimulated by glutamate alone, which is in vast contrast to mitochondria from normoglycemic controls. The glutamate-stimulated oxygen consumption by the diabetic mitochondria is prevented by either addition of GDP or removal of the free fatty acids, which further supports the conclusion of mitochondrial uncoupling. Although remaining to be supported by future experiments, we propose that the increased UCP-2 expression in the diabetic kidney is an antioxidant defense, which serves to reduce the mitochondria superoxide radical production and thereby preserve the function of the electron transport chain.

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S12/2 Pathogenic mutations in the mtDNA ATP6 gene and impairment of the ATP synthase energy transduction

Giancarlo Solaini, Gianluca Sgarbi, Gabriella Casalena, Giorgio Lenaz, Alessandra Baracca
Dipartimento di Biochimica, Università di Bologna, Italy
E-mail: giancarlo.solaini@unibo.it

We used different human cells to elucidate the molecular mechanism responsible for biochemical and clinical phenotypes associated with mutations at nt8993 in the mitochondrial DNA (mtDNA). The most common human mutations at this nucleotide, T>G and T>C, change Leu-156 for Arg and Pro, respectively, in the ATPase6 subunit (homologous to subunit *a* of *E. coli*) of the F_1F_0-ATPase (ATP synthase). When Pro substitutes for Leu, both clinical and biochemical phenotypes are rather mild, and ATP synthesis rate is found less than 20% reduced, matching the decrease of proton translocation rate through F_0 during ATP synthesis. At variance, the mtDNA 8993T>G mutation, bringing in the ATPase6 subunit Arg-156 and being associated with severe syndromes of infancy and childhood, induces a dramatic decrease of ATP synthesis rate, an impaired proton translocation rate, but an almost normal ATP hydrolysis rate. Taking into account the above observations, and on the basis of structural prediction analysis of mutant ATPase6 subunit, we suggest that the proton translocation pathway through F_0 is impaired by the T>G mutation, possibly due to partial loss of the Leu-156-containing transmembrane helix, which is essential for energy transduction by the ATP synthase. In conclusion, our study demonstrates the important role of Leu-156 for the ATPase6 structure allowing mitochondrial F_1F_0-ATPase energy transduction, and it provides a molecular mechanism for the pathogenesis of severe human syndromes. Moreover, our results suggest that mitochondrial

diseases associated with the only T>G mutation are primarily caused by a severe bioenergetic deficiency.

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S12/3 Recent progress in elucidating the molecular mechanism of the mitochondrial permeability transition pore

Andrew P. Halestrap
Department of Biochemistry and The Bristol Heart Institute, University of Bristol, UK
E-mail: A.Halestrap@Bristol.ac.uk

Opening of the mitochondrial permeability transition pore (MPTP) plays a key role in cell death, especially necrosis, and mediates the injury tissues such as the heart and brain experience following ischaemia and reperfusion. However, the molecular identity of the MPTP remains uncertain. Knockout studies have confirmed a role for cyclophilin-D (CyP-D), probably mediated by its peptidyl-prolyl *cis-trans* isomerase activity that facilitates a conformational change in an inner membrane protein. However, knockout studies have cast doubt on the central role of the adenine nucleotide translocase (ANT), previously implicated as the channel-forming component of the MPTP. The evidence for and against a role for the ANT in MPTP opening will be reviewed and data presented to suggest that it usually plays a regulatory role rather than provide the transmembrane pore component. Our recent data suggest that the protein fulfilling the latter role is the mitochondrial phosphate carrier (PiC) and recent evidence in support of this proposal will be summarised. Our data are consistent with a model for the MPTP in which a calcium-triggered conformational change of the PiC, facilitated by CyP-D, induces pore opening. We propose that this is enhanced by an association of the PiC with the "c" conformation of the ANT. Agents that modulate pore opening may act on either or both the PiC and the ANT.

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S12/4 Mitochondria as ATP consumers: the cell biology of the endogenous inhibitory protein, IF1

Michelangelo Campanella, Choon Tan, Andrey Abramov, Andrew Tinker, Michael Duchen
Departments of Physiology and Medicine, UCL, London WC1E6BT
E-mail: m.duchen@ucl.ac.uk

When the mitochondrial membrane potential ($\Delta\psi_m$) is compromised, the F_1F_oATP synthase runs in reverse, and mitochondria switch from ATP producers to consumers. In studies of the isolated enzyme, the protein IF_1 inhibits ATPase activity at an acidic pH. As its impact on mitochondrial function in intact cells is not established, we have explored the effect of overexpression or knockdown of IF_1 in cell lines (HeLa, C2C12). Upon inhibition of respiration, IF_1 conserves ATP (measured using luciferase transfected cells or measurements of $[Mg^{2+}]_c$) at the expense of mitochondrial depolarisation and reduces hypoxic cell death. Knocking down the protein promoted conservation of $\Delta\psi_m$ at the expense of ATP. Surprisingly, IF_1 also had a profound impact on mitochondrial structure and function: IF_1 overexpression increased both the number of mitochondrial cristae and ATP synthase activity, decreasing $\Delta\psi_m$ and favouring a dependence of ATP homeostasis on oxidative phosphorylation. Knocking down the protein had the opposite effect. Further, using immunofluorescence, we found that the relative expression of IF_1 to ATPase is considerably greater in primary neuronal cultures compared to adjacent astrocytes, showing that IF_1 expression level is not fixed in relation to the ATPase. These observations show that IF_1 has an influence on mitochondrial function at rest and that it is effective at preserving cellular ATP in hypoxic or ischaemic conditions.

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S12/5 Mitochondrial glutamate pathways and the control of metabolic homeostasis

Pierre Maechler
Department of Cell Physiology and Metabolism, Geneva University Medical Centre, 1 rue Michel-Servet, 1211 Geneva 4, Switzerland

Cellular glutamate pathways are essentially controlled by mitochondrial metabolism. Although enzymes of the mitochondrial matrix have been studied quite extensively, the regulation of mitochondrial membrane carriers is still rather mysterious. Moreover, recent advances show that it is inappropriate to extrapolate regulation models acquired from one cell model to another, as every tissue uses glutamate for specific functions. Very little is known about molecular mechanisms responsible for tissue specificities. For instance, expression of different isoforms of glutamate carriers might contribute to tissue specificity. Regarding glutamate dehydrogenase, flux direction depends on metabolic parameters such as substrate availability, redox and energy state of mitochondria. These parameters may be tissue specific. At the post-translational level, new modes of regulations have been described these recent years. Indeed, ADP-ribosylation of GDH mediated by SIRT4 offers another regulatory mechanism that might be tissue specific, pending different levels of *SIRT4* expression. This newly identified mode of regulation certainly deserves further investigations to better integrate molecular and cellular glutamate pathways into metabolic homeostasis at the organism level.

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S12/6 Activity of uncoupling protein-2 in pancreatic beta cells

Charles Affourtit, Martin D. Brand
MRC Dunn Human Nutrition Unit, Cambridge, UK
E-mail: ca@mrc-dunn.cam.ac.uk

Pancreatic beta cells secrete insulin when blood glucose levels are high. Dysfunction of this glucose-stimulated insulin secretion (GSIS) is partly responsible for the manifestation of type 2 diabetes, a metabolic disorder that is rapidly becoming a global pandemic. Mitochondria play a central role in GSIS by coupling glucose oxidation to production of ATP, a signal that triggers a series of events that ultimately leads to insulin release. Beta cells express a mitochondrial uncoupling protein, UCP2, which is rather surprising as activity of such a protein is anticipated to lower the efficiency of oxidative phosphorylation, and hence to impair GSIS. The mounting evidence demonstrating that insulin secretion is indeed blunted by UCP2 agrees with this prediction, and has provoked the idea that UCP2 activity contributes to beta cell pathogenesis and development of type 2 diabetes. Although this notion may be correct, the evolved function of UCP2 remains unclear. In this lecture, data will be presented that were obtained from our RNA interference studies to probe the effect of *Ucp2*