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

Acknowledgments

This work was supported by Comitato Telethon Fondazione Onlus, Roma, project number GP0280/01 and by the grant PRIN-2006050378_003 of MUR, Roma.

doi:10.1016/j.bbabio.2008.05.297

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

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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.

doi:10.1016/j.bbabio.2008.05.298

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

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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.

doi:10.1016/j.bbabio.2008.05.299

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

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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.

doi:10.1016/j.bbabio.2008.05.300

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

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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*

knockdown on respiration and glucose-stimulated insulin secretion in INS-1E insulinoma cells. Furthermore, a model will be proposed that predicts a role for UCP2 in the coordination of the physiological response of beta cells to fluctuating nutrient supply.

doi:10.1016/j.bbabio.2008.05.301

(S12) Mitochondria and disease symposium abstracts (poster and raised abstracts)

S12.7 The use of oxygen and pH-sensitive fluorescent probes for the investigation of perturbed cell metabolism

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In standard in vitro systems, the main sources of cellular ATP are glycolysis and oxidative phosphorylation. The balance between these two related energy generating systems can therefore inform on disease states and toxicities where perturbed metabolism is implicated. A highly informative approach to the investigation of such metabolic perturbations is to measure both cellular oxygen consumption and extracellular acidification rate (ECA). Analysis of oxygen consumption gives specific information on OxPhos, while measurement of ECA, under the appropriate conditions, provides information on non-aerobic metabolism. Here we present a new method of assessment of ECA of both adherent and suspension cells, using a long-decay pH-sensitive fluorescent probe and convenient 96-well plate format. As probe emission lifetime is used as the readout, a simple transformation, allows the generation of ECA rates in units of [H+] per unit time. We also outline how these probes may be combined with the MitoXpress oxygen consumption assay providing a highly informative dual-parameter metabolic analysis. Such parallel measurements allow inferences to be drawn regarding the site of an observed metabolic insult; thereby allowing altered glycolytic activity to be delineated from direct mitochondrial effects. We examine how such an analytical approach may be deployed for the examination of the perturbed metabolism using compounds of known metabolic impact including antimycin, 2-deoxyglucose, oxamic acid and dichloroacetate as models. This analysis is then extended to more relevant models analyzing the effect of a panel of biguanides on cellular metabolism and relating these observations to their proposed mechanism of action.

doi:10.1016/j.bbabio.2008.05.302

S12.8 Glycine prevents mitochondrial impairment caused by left carotid occlusion

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Mitochondria play a sufficient role in neuronal function. Several cerebral disorders like a stroke results in neuronal degeneration and associated with substantial reduction in mitochondrial functional activity and apoptosis induction. Monitoring of mitochondrial capacity for oxidative phosphorylation could be used as an indicator of stroke development. There is evidence from clinical studies that glycine reduced brain damages caused by stroke. However, the mechanism of the protection afforded by glycine is not yet known. In the present study we attempted to elucidate the mechanisms of glycine anti-stroke activity. The left carotid artery occlusion was used as a model of brain ischemia. After occlusion respiratory control index of brain cortex mitochondria was measured. It was reduced from 6.7± 0.1 to 4.2±0.1 after 24 h occlusion. The development of apoptosis process was also detected, DNA internucleosomal fragmentation and caspase-3 activation was observed. When animals were treated with glycine *per os* before occlusion the reduction of respiratory ratio and caspase-3 activation were prevented. Glycine allows mitochondria to maintain their respiratory activity in ischemic conditions. Our novel data indicate that anti-stroke glycine activity is associated with its ability to prevent mitochondrial disorder and apoptosis development in brain cortex tissue induced by ischemia.

doi:10.1016/j.bbabio.2008.05.303

S12.9 Dynamic regulation of UCP2 concentration in INS-1E pancreatic beta-cells

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Uncoupling protein 2 (UCP2) is known to exacerbate the diabetic phenotype by diminishing glucose-stimulated insulin secretion (GSIS). It does this by uncoupling substrate oxidation from ATP synthesis; this uncoupling ultimately attenuates exocytosis of insulin-containing granules. In contrast, UCP2 downregulation improves GSIS and ameliorates diabetes.

UCP2 content in cells appears to be regulated at both the transcriptional and translation levels. We measured UCP2 content by Western blot in INS1E pancreatic beta-cells following addition of cycloheximide to inhibit protein synthesis and observed that UCP2 has a short half-life of about 1 h. To explore this dynamic regulation, we characterised endogenous UCP2 concentrations at the message and protein levels in this pancreatic beta-cell system. We found that over a 24 h period, depending on the concentrations of transcriptional and translational regulators (glucose, serum, glutamine) in the incubation medium, UCP2 protein concentration was 78–432 pg per 100,000 cells (2.6–13.4 ng/mg mitochondrial protein).

doi:10.1016/j.bbabio.2008.05.304

S12.10 Role of mitochondrial DNA mutations in periodontitis

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Periodontitis is an inflammatory disease affecting the tissues that surround the teeth, as a result of complex interactions between pathogenic bacteria and the host's immune response. There are increasing evidences on the implications of reactive oxygen species (ROS) in the pathogenesis of inflammatory disorders. Therefore, we aimed to analyze the role of genetics, biochemical, membrane potential and evolutionary background in periodontitis. We have