S4.29 Catalytic properties of Na^+-translocating NADH:quinone oxidoreductases from *Vibrio harveyi*, *Klebsiella pneumoniae*, and *Azotobacter vinelandii*

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The main goal of our study was a comparative analysis of the catalytic properties of sodium-translocating NADH:quinone oxidoreductases (Na^+-NQRs) from marine bacterium *Vibrio harveyi*, enterobacterium *Klebsiella pneumoniae*, and soil microorganism *Azotobacter vinelandii*. It is shown that their enzymes drastically differ in their affinity to sodium ions with apparent K_M values 2.7 mM, 0.67 mM and ≈0.1 mM respectively. The enzymes also possess different sensitivity to inhibitors. Na^+-NQR from *A. vinelandii* is not sensitive to low HQNO concentrations, while Na^+-NQRs from *V. harveyi* and *K. pneumoniae* can be inhibited with $I_{0.5}$ values 0.13 μM and 0.55 μM respectively. Also Na^+-NQR from *K. pneumoniae* is fully resistant to either Ag^+ (which is considered to be specific inhibitor of Na^+-NQR from *V. harveyi*) or *N*-ethylmaleimide. Na^+-NQR from *A. vinelandii* possess transitional sensitivity to these modificators of SH-groups. All the Na^+-NQR-type enzymes are sensitive to diphenyliodonium. So the main unique characteristic of Na^+-NQR is its specific requirement for sodium ions, which can be not readily detectable, since the affinity of Na^+-NQR to Na^+ can be very high.

doi:10.1016/j.bbabio.2008.05.160

(S5) Mitochondrial biogenesis symposium lecture abstracts

S5/1 Control of the synthesis of uncoupling and coupling proteins in brown adipose tissue

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In active brown adipose tissue, the balance between coupled and uncoupled respiration is the opposite of that seen elsewhere. This is accomplished through two features: low content of coupling proteins (the ATP-synthase complex) and high content of uncoupling protein (UCP1). In the tissue, very high expression (mRNA levels) of all subunits of ATP-synthase is seen — except for subunit c, implying that ATP-synthase assembly is under control of sub-c amount. We have now demonstrated that artificially-induced overexpression of sub-c results in increased amounts of fully competent ATP-synthase. In wildtype, despite high mRNA levels for the other subunits, no unassembled ATP-synthase subunits are observed in blue-native gels. This implies translation control of the other components of the ATP-synthase. Concerning the β-subunit, the control in different tissues may be related to formation of an RNA/protein complex that is dependent on a stem–loop structure in the 3′UTR mRNA. Brown adipose tissue recruitment and UCP1expression are normally considered to be under sympathetic control. There is physiological reason for a nonsympathetic recruitment pathway. We find that chronic treatment of brown (pre)adipocytes with PPARγ-agonists activates mitochondriogenesis and UCP1 expression, leading to thermogenically competent brown-fat cells, i.e. cells that although naive to norepinephrine respond thermogenically to norepinephrine.

doi:10.1016/j.bbabio.2008.05.161

S5/2 Regulation of mitochondrial dynamics by nitric oxide is a key event in myogenesis

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Mitochondria exist in two interconverting forms, i.e. as small isolated particles, and as extended filaments, networks or clusters. Here we provide evidence that in differentiating myoblasts endogenous nitric oxide (NO) generation controls mitochondrial shape: in the absence of NO mitochondrial fission occurs rapidly. The action of NO is specifically addressed to mitochondrial fission since in PEG fusion assay organelle fusion was not modified by the treatment with the NO synthase-inhibitor L-NAME. A key protein involved in mitochondrial fission is the large GTPase DRP-1. DRP-1 translocation to the mitochondria promotes mitochondrial fission. DRP-1 translocation and mitochondrial fission were stimulated by L-NAME and inhibited by exogenous NO. In addition, NO inhibited DRP-1 GTPase activity. We also found that in differentiating myoblasts NO is required for the expression of differentiation markers including myogenin and muscle specific myosin since L-NAME inhibited myogenic differentiation, and exogenous NO restored it. Overexpression of a dominant negative DRP-1 reversed the inhibitory effect of L-NAME on myogenesis. Our results indicate that NO controls a key event in mitochondrial dynamics that may have relevant implications for both myogenesis and control of energy metabolism in developing skeletal muscle.

doi:10.1016/j.bbabio.2008.05.162

S5/3 Bioenergetics of mitochondrial protein topogenesis

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The TIM23 translocase is involved in the topogenesis of the vast majority of mitochondrial proteins. Preproteins pass through the TOM complex of the outer membrane and are then transferred across or laterally inserted into the inner membrane (IM). The electrical membrane potential $\Delta\psi$ is required for the translocation of the targeting signal across the IM where it can be reached by the chaperone mtHsp70. Further translocation does not require $\Delta\psi$, but instead matrix ATP. ATP hydrolysis drives cycles of binding of mtsp70 to incoming unfolded preproteins. mtHsp70 is part of the mitochondrial import motor which comprises further components,

(i) Tim44, a coordinating platform, (ii) the subcomplex Tim14–Tim16, J and J-like proteins regulating the ATPase of mtHsp70, and (iii) Mge1 exchanging ADP vs. ATP on mtHsp70. Structure determination of the Tim14–Tim16 oligomer led to a working hypothesis for the import motor: the Tim14–Tim16 pair switches between two conformations, one in which the HPD motif of Tim14 is available for activating the ATPase domain of mtHsp70 and another one in which the activation is blocked. The switch of the TIM14–Tim16 pair is linked to changing interactions with Tim44 and mtHsp70. The reactions of the various components of the import motor are consistent with a Brownian ratchet type mechanism. In this model, spontaneous oscillations of the unfolded preprotein chain in the import channels of TOM and TIM23 complexes are converted into unidirectional movement by preventing retrograde sliding by regulated transient binding of mtHsp70.

doi:10.1016/j.bbabio.2008.05.163

(S5) Mitochondrial biogenesis symposium abstracts (poster and raised abstracts)

S5.4 Mitochondrial function in cancer cell line models

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Mitochondrial function and respiratory activity is regulated via complex mechanisms that respond to energy status, metabolic elements and stress insults. Elements of mitochondrial dysfunction have been connected to diseases such as metabolic syndromes, cancer and degenerative disorders. The search for biomedical modalities for improvement or stimulation of mitochondrial oxidative activity is therefore considered to be an attractive approach for finding new therapeutic procedures.

The objective of this study was to investigate effects in a selection of cancer cell lines exposed to agents expected to increase mitochondrial biogenesis and respiration. We used pharmacological modulators of glycolysis, respiration and energy status to selectively invoke and facilitate a metabolic shift in the cells towards increased mitochondrial oxidative phosphorylation. Parameters such as cellular content of mitochondria, mitochondrial membrane potential, respiratory rate and glycolytic activity were then analysed. The cell lines exposed different metabolic effects of the treatment, and some cell models were less tolerant than others since the viability was reduced. The metabolic flexibility of the cells seemed to be connected to their ability to thrive under these conditions. This demonstrates that metabolic modulation may have consequences for cell growth and survival, and such approaches may therefore be useful in cancer therapy. Tumours do normally have increased rates of glycolysis combined with reduced respiratory activities, and by targeting this feature it might be possible to develop more selective therapeutic approaches for tumours of different origins.

doi:10.1016/j.bbabio.2008.05.164

S5.5 The trehalose pathway regulates mitochondrial respiratory chain content through hexokinase2 and AMPc IN *Saccharomyces cerevisiae*

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TPS1 encodes for the trehalose-6-P synthase. The trehalose-6-phosphate phosphatase is encoded by TPS2. We studied the respiratory metabolism of both Δtps1 and Δtps2 strains. We show that mutants of the trehalose pathway exhibit modification in the respiratory chain content. In the Δtps1 there is a decrease in the amount of respiratory chains within the cells whereas in the Δtps2 there is an increase in this amount. Because the mitochondrial enzymatic content is modulated through the activity of the Ras/PKA/cAMP pathway, we assessed cAMP content in these strains. There is a good positive correlation between the cellular cytochrome $a+a_3$ content and the cellular cAMP amount. Thus, the effect of the mutations in the trehalose synthesis pathway on mitochondrial enzymatic content is mediated by cAMP level. Furthermore, we investigated the consequences of such mutations on hexokinase 2 deleted strains. In all three hexokinase deleted strains, the mitochondrial amount is comparable to the wild type. Thus, the influence of the tps1 and tps2 deletions on cAMP levels are likely to go through hexokinase.

doi:10.1016/j.bbabio.2008.05.165

S5.6 Reactive oxygen species mediated down-regulation of mitochondrial biogenesis

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Mitochondrial biogenesis necessitates the participation of both the nuclear and the mitochondrial genomes. It is highly regulated and mitochondrial content within a cell varies according to energy demand. In the yeast *Saccharomyces cerevisiae*, the cAMP pathway is involved in the regulation of mitochondrial biogenesis. An overactivation of this pathway leads to an increase in mitochondrial enzymatic content. Out of the three yeast cAMP protein kinases, we have shown that Tpk3p is the one involved in the regulation of mitochondrial biogenesis. Moreover, in the absence of Tpk3p, mitochondria produce large amounts of reactive oxygen species (ROS) that signal to the HAP2/3/4/5 nuclear transcription factors. These transcription factors are well-known to be involved in mitochondrial biogenesis. We clearly establish that an increase in mitochondrial ROS production down-regulates mitochondrial biogenesis. Furthermore, we identified the cysteine of the HAP4 transcription factor that serves the role of sensor of these ROS and is crucial for this signaling. It is the first time that a reactive oxygen specie sensitivity of the transcription factors involved in yeast mitochondrial biogenesis is shown. Such a process could be seen as a mitochondria quality-control process.

doi:10.1016/j.bbabio.2008.05.166

S5.7 Respiratory chain organization in *Neurospora crassa* upon disruption of mitochondrial bc_1 complex

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