7L.3 Shotgun proteomics reveals a mitochondrial function for the BK channel in cochlea

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Large conductance Ca^{2+}-activated K^+ (BK) channels regulate important physiological processes, such as neuronal excitability, signal transduction, metabolism, and immunity. Thus, this channel will partner with a number of different types of proteins. We identified 174 BK-associated proteins (BKAPs) in mouse cochlea (Kathiresan *et al.*, 2009 *Mol. Cell. Prot.* 8: 172–187) and, more recently, 125 in chick using BK coimmunoprecipitation (coIP), 2-D gel electrophoresis, and LC-MS/MS. Using the primary partners of BK, the molecular interaction database IntAct (www.ebi.ac.uk) (Kerrien *et al.*, 2007, *Nucl. Acids Res.* 35: 561–565) was searched for putative secondary partners. Binary partners were determined using Envision tool, www.ebi.ac.uk/enfin-srv/envision, with the search limited to murine proteins and physical interactions, that is, excluding cosedimentation data. Interaction networks (interactome) were visualized, modelled, and analyzed using Cytoscape (Cline *et al.*, 2007, *Nat. Protoc.* 2: 2366–2382). The analysis for mouse revealed 13 networks involving 199 proteins and 254 interactions. Of these proteins, 160 are nodes (proteins) linked with 188 edges (lines connecting interactions) to form a single global network. The analysis for chick revealed 19 networks involving 190 proteins and 189 interactions. Of these proteins, 119 are nodes linked with 136 edges to form a single global network. Another analysis was undertaken to determine which pairs (primary with secondary partners) of BK interactions are conserved across different species by searching the NCBI KOG database. An iKOG identity was given to each protein using the STRING 8.1 database to generate interacting clusters of orthologous groups (iKOGs). Results were visualized by producing a matrix of the iKOGs across six different species using heatmap.plus v1.3. Profiles from these analyses separated into distinct functional conservation clusters that consisted of proteins involved in transport, calcium binding, chaperoning, and signal transduction, while the interactome revealed clusters of BKAPs localized to the nucleus, ER, and mitochondria. Immunofluorescence, using cochlear tissues, showed BK expression in the mitochondria of the sensory cells. Given these data, we have begun to verify the BK interactome using siRNAs in heterologous expression systems such as CHO cells.

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7L.4 Novel applications of blue-native PAGE

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Blue-native electrophoresis is one of the most popular techniques to isolate native membrane protein complexes for a variety of biochemical and clinical purposes and especially for the estimation of native protein masses. Non-ideal mass markers and acrylamide gels, however, can compromise the accuracy and reliability of native mass estimations. Here we discuss the principles and general rules for the determination of mass and oligomeric state of native membrane and soluble proteins. Using mild detergents for membrane solubilization and native electrophoresis for protein separation even labile supramolecular assemblies like respiratory chain supercomplexes and oligomeric ATP synthases can be isolated. The complexity of these higher order structures can be reduced for proteomic investigations by applying less mild native electrophoresis variants in the second dimension. Supercomplexes thereby dissociate into individual complexes. Clear-native and blue-native electrophoresis variants are useful alternatives for the second native dimension, but clear-native electrophoresis is advantageous for the identification of fluorescence-labeled proteins and for in-gel activity assays that commonly interfere with the Coomassie-dye. We expand the application of blue native electrophoresis to the separation of mega protein complexes to the mass range of 10–50 MDa by introducing novel large pore acrylamide gels. The novel gel types were then used to search for suprastructures of mitochondrial respiratory supercomplexes, the hypothetical respiratory strings or patches [1–3].

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Posters

7P.1 Gene identification and functional characterisation of *Plasmodium falciparum* succinate dehydrogenase

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Mitochondrial succinate dehydrogenase (succinate:quinone oxidoreductase, Complex II) is a key component of both the respiratory electron transfer chain and the tricarboxylic acid cycle, and presents itself as a potentially attractive antimalarial chemotherapeutic target. Eukaryotic Complex II is an integral membrane protein, consisting of four subunits. Despite quantifiable succinate:quinone oxidase activity in *P. falciparum* membrane preparations (which we demonstrate here for the first time), the identity of two Complex II subunits (SdhC and SdhD) remains obscure in the human malaria parasite genome sequence data. By using a bioinformatic analysis based on a structural filtering of all genes from PlasmoDB database, we identified candidates for SdhC and SdhD. Despite the poor cross-species sequence conservation of SdhC and SdhD, our proteins are predicted to exhibit convincing structural and functional homology with the elucidated crystal structures of Complex II. To examine the validity of the SdhC and SdhD candidates, we present a proteomic analysis of *P. falciparum* mitochondrial membrane fractions based on immunocapture with a cross-species antibody against Complex II followed by Liquid Chromatography Mass Spectrometry (LC-MS).

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7P.2 New insights in the mitochondrial localization of APE1, the major apurinic/apyrimidinic endonuclease in mammals

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Apurinic apyrimidinic endonuclease 1/redox effector factor 1 (APE1/Ref-1) is a vital protein that acts as a master regulator of cellular response to oxidative stress conditions and contributes to the maintenance of genome stability [1, 2]. APE1 is involved in both the base excision repair (BER) pathways of DNA lesions, acting as the major apurinic/apyrimidinic (AP) endonuclease, and in transcriptional regulation of gene expression as a redox coactivator of different transcription factors. In different mammalian cell types the APE1 subcellular distribution is mainly nuclear and is critical for controlling cellular proliferative rate [3]. However, cytoplasmic, mitochondrial, and endoplasmic reticulum localization has also been reported [4] but the specific mechanism for redirecting APE1 into the different subcellular compartments is not known. Mitochondrial DNA is constantly exposed to oxidative injury. Due to its location close to the main source of reactive oxygen species, the inner mitochondrial membrane, mtDNA is more susceptible than nuclear DNA to oxidative damage. Because BER is the main DNA repair pathway coping with oxidative lesions, most of the investigations on mitochondrial DNA repair focus on mtBER. To verify mitochondrial localization of APE1/Ref-1, a flag-tagged protein was expressed in HeLa cells and its intracellular localization was tracked by immunocytochemistry. Subfractioned bovine heart mitochondria were also used to analyze the localization of mtAPE1. Mitoplasts were prepared by low digitonin treatment of mitochondria and separated from fraction containing outer membranes and intermembrane space soluble proteins by differential centrifugation. After submitochondrial fractionation, mtAPE1 was predominantly found in the soluble protein rich fraction of the intermembrane space. Notably, in agreement with a previous study [5], only the full-length protein was detected in mitochondrial extracts, raising doubts about the mitochondrial targeting mechanism previously suggested [6]. The mitochondrial import pathway and the potential targeting signals of mtAPE1 are currently under investigation.

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7P.3 Effects of dietary methylmercury on the Zebrafish (*Danio rerio*) liver proteome expression and mitochondrial function

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In the present work we have studied the chronic and the acute effects of methylmercury (MeHg) in a model fish (*Danio rerio*). The chronic effects of methylmercury intoxication were showed by a comparative proteomics approach realized on the cellular and mitochondrial soluble proteome in the liver. At the mitochondrial proteome level 56 proteins exhibiting a significant difference in their abundance were identified. The proteins involved in the Krebs cycle and the amino acid metabolism were down-regulated while the enzymes implicated in the β-oxidation and the OXPHOS apparatus were up-regulated as well as the antioxidant systems and the ROS scavenger enzymes. At the cellular proteome level, 74 proteins were identified and exhibited a significant difference. The results suggested that glycolysis is down-regulated, as well as the pentose phosphate pathway. The acute effect of methylmercury was studied on isolated liver mitochondria using a Clark electrode oxymeter. The state 3 mitochondrial respiration was readily inhibited by MeHg, on the contrary the state 4 respiration exhibited a concentration threshold in the inhibition, suggesting an inhibition at the level of the dicarboxylate transport. This proposal was confirmed by comparing the effects of both n-butylmalonate, a well-known inhibitor of the dicarboxylate carrier, and MeHg. Thus the proteomic approach allows us to understand the metabolic changes that permit the cell to ensure its viability during dietary methylmercury intoxication. According to respiratory results it could be proposed that those changes were the consequences of substrate transport impairment by MeHg.

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7P.4 Src-mediated tyrosine phosphorylation: A new way of regulating mitochondrial bioenergetics?

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Mitochondria produce the most part of the energy consumed by cells through oxidative phosphorylation. Since energetic demands of cells depend on their functions, energy production must be tightly regulated. Phosphorylation (or dephosphorylation) is one of the major regulation pathways of enzymes. Numerous proteins are tyrosine-phosphorylated among electron transport system components in rat brain mitochondria [1]. Several tyrosine kinases are also present in those mitochondria but the tyrosine Src kinase seems the most active of them [2]. Our goal was to study how the regulation of this kinase influences oxidative phosphorylation in rat brain mitochondria. First, we demonstrated that tyrosine phosphatase PTP1B, a well-known Src regulator in cytosol [3], seems to regulate Src activity in rat brain mitochondria as it does in cytosol (i.e. it activates Src by dephosphorylating its terminal pY527 residue). Moreover, *in vitro* ATP addition caused activation of Src and global increase of tyrosine-phosphorylated mitochondrial proteins. The global increase of tyrosine-phosphorylated mitochondrial proteins was accompanied by an increase of COX activities and decrease of complexes III and V. Besides, activation of Src, increase of tyrosine phosphorylation and changes of enzymatic activities were reduced when mitochondria were preincubated with pharmacological inhibitors of Src or PTP1B. Finally, mitochondrial ADP-stimulated respiration decreased in the presence of each inhibitor. These results clearly show that Src kinase activity (partly regulated by PTP1B) influences mitochondrial physiology. Therefore, Src-mediated tyrosine phosphorylation seems to represent a major regulation pathway of oxidative phosphorylation, at least in rat brain mitochondria.

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