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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