Temperature dependent steady state NOE experiments and NMR linewidth measurements indicate increased molecular motion in the EF-hand consistent with a proposed role for PC2-EF as a Ca2+-sensitive regulator. Structure-based sequence conservation analysis reveals a conserved hydrophobic pocket in this region, where PC2-EF may mediate Ca2+-dependent protein interactions. Using results of our structural studies we have examined the role of the EF-hand and coiled coil on PC2 channel function in single-channel lipid bilayers. Our results suggest that the coiled coil regulates PC2 by serving as an homo-oligomerization motif, whereas the EF-hand modulates the Ca2+-dependence of PC2 channel activity. Based on our results we propose a mechanism of regulation of the Ca2+-dependence of PC2 channel activity by PC2-EF.

2751-Pos

New Channels in the Outer Mitochondrial Membrane

Vivien Krüger[1], Michael Meinecke[2], Lars Becker[3], Chris Meisinger[4], Nikolaus Pfanner[4], Richard Wagner[1].
[1]University of Osnabrueck, Osnabrueck, Germany, [2]LMB Cambridge, Cambridge, United Kingdom, [3]University of Stanford, Stanford, CA, USA, [4]University of Freiburg, Freiburg, Germany.

Mitochondria are the "power stations" of eukaryotic cells. Beside this they play essential roles for the metabolism and physiology of cells and are a central point of apoptosis regulation. Mitochondria are also involved in calcium homeostasis. Due to endosymbiontic engulfment mitochondria are surrounded by two membranes. While the regulation of the metabolite flux across the inner membrane (IMM) is extensively characterised, it has been generally assumed that the outer membrane (OMM) functions only as a barrier for molecules larger than 3 kDa. But recent studies demonstrate that the metabolite flux between the cytosol and the different compartments of mitochondria is regulated at the level of the outer membrane.

Three pore forming proteins are up to now known in the outer membrane. Two of them are essential and involved in protein transport and insertion into OMM. These are Tom40 and Sam50/Tob55. The third one is the non-essential metabolite pore VDAC (voltage-dependent anion channel). The none lethal phenotype of VDAC knockouts discloses that it is the sole metabolite conducting pore in the OMM and the presence of other non-identified channels in the OMM is very likely.

The OMM proteome contains more than 112 proteins and only for less than 10 % of them the function is known. By electrophysiological screening of highly pure $OMM_{vdac\ \Delta}$ vesicles it was possible to identify at least four distinct membrane pores. In a first bioinformatical attempt using specific parameters like the isoelectric point or second structure prediction programs we identified eight potential channel candidate proteins.

2752-Pos

Evidence for Lateral Budding and Voltage Dependence of a Proteo-Lipid Channel

Debra Datskovskiy, **Meenu N. Perera**, Megan Miles, Toan Nguyen, Marco Colombini.
University of Maryland, College Park, MD, USA.

The pro-apoptotic protein, Bax, and the sphingolipid, ceramide, can individually form channels in phospholipid membranes. When combined, they permeabilize membranes in a synergistic way, indicating the formation of a combined channel structure. Nanomolar quantities of $LaCl_3$ disassemble ceramide channels completely but 10 micromolar $LaCl_3$ is needed to convert one large Bax-ceramide channel into a population of virtually identical channels. These channels exhibit voltage-dependent closure or disassembly. Some of the channels can be reassembled by reducing the voltage or applying an opposite potential but cycles of voltage-dependent closure and reopening quickly result in loss of conductance. There are indications that the transformation of the one large channel into a population of small channels occurs by lateral budding in the plane of the membrane. Over 100 such small channels were formed in one experiment and the application of an elevated potential resulted in a long staircase of virtually identical conductance decrements. These results open a window into phenomenology that, to our knowledge, has not been described previously.
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2753-Pos

A Kinetic Model of Ion Channel Electrophysiology: Incorporating Bilayer-Mediated Effects of Agonists and Anesthetics on Protein Conformational Transitions

Robert S. Cantor, Kathryn S. Twyman, Daniel J. Albershardt.
Dartmouth College, Hanover, NH, USA.

The time- and concentration dependence of agonist-induced ion currents through postsynaptic receptors is often remarkably complex, involving desensitization and deactivation on multiple time scales, as is the modulation of these currents by other solutes such as anesthetics. Traditional kinetic models have involved agonist binding and conformational transitions among a very large manifold of protein conformational states engineered to reproduce the complexity of a particular set of electrophysiological results. However, independent experimental evidence for the hypothetical additional conformational states (beyond the minimal set of resting, conducting and desensitized) is essentially nonexistent, nor is there any model-independent way of estimating the values of the associated kinetic parameters. We propose an alternative model that includes only these three essential states while additionally incorporating the adsorption of agonist and nonbinding compounds such as anesthetics to the bilayer in which these intrinsic membrane proteins are embedded [R. S. Cantor et al., *Soft Matter*, 2009, **5**, 3266]. Solute adsorption alters bilayer physical properties, which in turn distorts the protein conformational free energy landscape, and thus alters the rate constants of protein conformational transitions. The complexity of the predicted ion currents - often well approximated as sums of exponentials - then arises from the time-dependence of solute adsorption, resulting in strongly time-dependent transition rate ''constants''. If only nonbinding solutes are present, the model simplifies considerably. For this special case, best fits of predicted current traces with respect to a small set of parameters are in excellent agreement with fast-perfusion electrophysiological studies of recombinant $GABA_A$ receptors [R. Haseneder et al., *Eur. J. Pharm.*, 2002, **451**, 43] in which currents are induced in the absence of agonist by a broad range of supraclinical concentrations of isoflurane and sevoflurane.

2754-Pos

KChiP2 Stabilizes Kv4 Protein Expression and Cell Surface Retention to Control Cardiac Ito Channel Densities

Nicholas C. Foeger[1], Céline Marionneau[2], Jeanne M. Nerbonne[1].
[1]Washington University in St. Louis, St. Louis, MO, USA, [2]Institut du Thorax, Nantes, France.

The myocardial transient outward current (I_{to}) is encoded by voltage-gated potassium (Kv) channel α-subunits of the Kv4 subfamily, together with the cytosolic accessory subunit, KChIP2. Targeted deletion of KChIP2 (KChIP2-/-) or Kv4.2 (Kv4.2-/-) eliminates I_{to} in adult mouse ventricular myocytes. Heterologous co-expression with KChIP2 increases Kv4.2 current densities and results in a relative shift in Kv4.2 from a perinuclear localization to the cell surface, leading to the suggestion that KChIP2 alleviates retention of assembled Kv4 channels in the endoplasmic reticulum (ER) and promotes forward trafficking. To explore these hypotheses, a putative RXR-type ER-retention motif at residues 35-RKR-37 in Kv4.2 was mutated (Kv4.2AAA), and the functional consequences of this construct on Kv4.2 expression in human embryonic kidney-293 (HEK) cells were explored. Mean ± SEM peak Kv4.2 current densities in cells expressing Kv4.2AAA (316 ± 50 pA/pF) were significantly ($p=0.025$) higher than in cells expressing wild type Kv4.2 (174 ± 20 pA/pF). Surprisingly, however, adenoviral expression of Kv4.2AAA in Kv4.2-/- myocytes resulted in peak Kv current densities (86 ± 9 pA/pF) that were not significantly different from the peak Kv currents (72 ± 9 pA/pF) in Kv4.2-/- cells infected with wild type Kv4.2. Heterologous expression of a charge-conservative mutant, Kv4.2KKK, in which arginines 35 and 37 were mutated to lysines (Kv4.2KKK), resulted in Kv4.2 currents (172 ± 25 pA/pF) that were indistinguishable from wild type currents, demonstrating that the presence of charged residues in the Kv4.2 N-terminus affects channel gating, not channel trafficking. Biochemical studies revealed no differences in the surface expression of the Kv4.2AAA mutant and wild type Kv4.2, and the surface expression of both constructs was increased dramatically upon co-expression of KChIP2. The results of further biochemical studies suggest that KChIP2 functions to increase the retention of Kv4.2 channels at the cell surface.

2755-Pos

The Na^+-Activated Potassium Channel Slack Shares a Similar Na^+ Coordination Site with Kir3 Channels

Zhe Zhang[1], Avia Rosenhouse-Dantsker[2], Qiongyao Tang[1], Sergei Noskov[3], Diomedes E. Logothetis[1].
[1]Department of Physiology and Biophysics, School of Medicine, Virginia Commonwealth University, Richmond, VA, USA, [2]Department of Medicine, University of Illinois at Chicago, Chicago, IL, USA, [3]Institute of Biocomplexity and Informatics, University of Calgary, Calgary, AB, Canada.

Characteristics of Na^+ activated potassium channel (Slack or Slo2.2) currents, including high conductance, rundown, regulation by Na^+, Cl^- and phosphorylation have long been reported but underlying mechanisms remain unknown. Here we report identification of a sodium regulatory site in the RCK2 domain of Slack channels by screening the C-terminus with the conserved sodium coordination motif of Kir channels. While the charge preserving D818E mutation exhibited similar Na^+ sensitivity as the wild-type Slack channel, both