Cx26 (hCx26) channels seems to represent the channel with open gates. This structure, and molecular dynamics studies based on it, reveal that charged residues (D46, E47, R75, R184) at the extracellular entrance of the aqueous pore - a region thought to be involved in gating rearrangements - form an electrostatic network. We explored the role of these salt bridge interactions in gating using mutagenesis, kinetic analysis and chemical modifications. Substitution of neutral residues for D46 or E47, which would eliminate their participation in salt bridges, accelerate deactivation kinetics and moderately increase the apparent affinity of Ca^{2+} to induce channel closing. These data support a role of these residues in stabilization of the open state. In addition, when D46 is substituted by a cysteine (D46C), modification by MTSES to add a negative charge increases holding and tail currents. This suggests that a negative charge at this position is involved in stabilizing open hemichannels. In wild-type channels, following depolarizing pulses to 0 mV, peak tail currents increase as a function of pulse duration, reaching maximum with pulses of 40 sec. Strikingly, E47A/Q mutations showed peak tail currents that saturate more rapidly, at 15 sec, suggesting that this position also plays a key role in hemichannel activation. Thus far, our data suggest that intra- and inter-subunit electrostatic networks at the extracellular entrance of the hCx26 pore play critical roles in hemichannel gating reactions. Support: R01GM099490.

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Extracellular Divalent Cations Regulation of Cx26 and Cx30 Hemichannels

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Due to the large size and modest selectivity of the aqueous pore, exacerbated opening of connexin hemichannels leads to loss of electrochemical gradients and of small cytoplasmic metabolites, causing cell death. Control of hemichannel opening is indispensable, and is achieved by extracellular divalent concentrations, which drastically reduces hemichannel activity. Here, we explore the differences between extracellular Ca^{2+} and Mg^{2+} regulation in two relatives connexin, hCx26 and hCx30. Our standard protocol for assessment connexin hemichannel activation and deactivation with the two electro-voltage clamp technique is to examine the peak tail currents and their relaxation kinetics following a depolarizing pulse from −80 mV to 0 mV. using this protocol, the peak tail currents increase with reduction of external divalents. We estimate the extracellular Ca^{2+} and Mg^{2+} apparent affinity for hCx26 hemichannels at values of 0.33 mM and 1.8 mM, respectively. hCx30 hemichannels showed slightly higher extracellular Ca^{2+} and Mg^{2+} apparent affinity with values of 0.17 mM and 1.0 mM, respectively. At physiological Ca^{2+} concentration (1.0 - 1.8 mM), both hCx26 and hCx30 hemichannels reach $\leq$ 15% of the maximal response, but at corresponding Mg^{2+} concentrations they reach $\geq$ 50%. In addition, deactivation time constant at the tail currents are accelerated as a function of Ca^{2+} concentrations in hCx26 and hCx30 hemichannels; however, only high extracellular Mg^{2+} concentrations ($>$ 2.0 mM) are capable to accelerate deactivation kinetics in both connexin types. The holding currents at steady state are significantly increased at physiological extracellular Mg^{2+} concentrations (1.0 - 1.2 mM) in both hCx26 and hCx30 suggesting an increase in open hemichannels even at negative potentials. Our data support that, under physiological ionic conditions, Ca^{2+}, but not Mg^{2+} plays a major role stabilizing and facilitating closing of Cx26 and Cx30 hemichannels. Support: R01GM099490

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The Effect of Arachidonic Acid on Junctional Conductance and Gating of Connexin 36 Gap Junction Channels and their Modulation by N-Alkanols

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Arachidonic acid (AA) is one of major components of membrane phospholipids and is actively involved in the regulation of junctional conductance (g_j). *N*-alkanols are well known inhibitors of gap junction (GJ) channels and have been shown to reduce neurological tremors. In this study, we examined function of Cx36 GJ channels, which are expressed in neurons and β-cells of pancreas, under control conditions and application of factors modulating concentration of AA in the plasma membrane. We found that in *HeLa* cells expressing Cx36-EGFP, short carbon chain alkanols (SCCAs), such as pentanol, hexanol and heptanol, increased g_j by ~3-fold. Conversely, long carbon chain alkanols (LCCAs), such as octanol, nonanol and decanol, uncoupled cells fully. We demonstrate that under control conditions only ~0.003 of Cx36 GJ channels assembled in junctional plaques are functional, and this fraction increases by SCCAs and fatty acid free bovine serum albumin (BSA). BSA increased g_j by 1.6-fold with EC_{50} of 2.3 M, while its modified form 1,2-cyclohexanedione (BSA-CHD), which does not bind AA, was ineffective. Voltage sensitive gating of Cx36 GJs was reduced by SCCA and BSA that explain in part their g_j-enhancing effect. The inhibition of Cx36 GJ channels by AA can be rescued by BSA but not by BSA-CHD. MAFP and thapsigargin, inhibitor and activator of AA synthesis via phospholipase A_2, increased and reduced g_j, respectively. We assume that endogenous AA is one of key factors leading to low functional efficacy of Cx36 GJ channels under control conditions. Furthermore, we suggest that g_j-enhancing effect of BSA and MAFP may be related with reduction of AA levels, while SCCAs limit AA's accessibility to its binding site on Cx36.

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Ion Channels Formed by SARS Coronavirus Envelope Protein: Lipid Regulation of Conductance and Selectivity

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Coronaviruses (CoV) are pathogens that cause common colds, bronchiolitis and acute respiratory distress syndrome. In fact, their relevance increased when the causative agent of the severe acute respiratory syndrome (SARS) was identified as a CoV. CoV E protein is a small transmembrane protein of between 76-109 amino acids in length that modulates coronavirus morphogenesis, tropism and virulence [1].

We have reported that E protein conductance and ion selectivity were controlled by the lipid composition of the membrane [2]. These results indicated that, most likely, lipid molecules assembled with the peptide oligomers to form the channel.

Here we provide additional evidences of the functional involvement of lipids in the channel structure. The influence of lipid molecules on E protein channel transport properties was investigated focusing on the salt concentration dependence of the E protein conductance and the pH dependence of the channel ion selectivity.

The channel conductance in neutral bilayers increased with the electrolyte concentration whereas in charged bilayers it is approximately proportional to the square root of salt concentration, which reveals an electrostatic contribution from the lipid charge. In regard to pH dependence of ion selectivity, in uncharged bilayers the titration curve shows a single transition that corresponds to E protein residue titration, whereas in charged bilayers a second transition is observed, which presumably corresponds to lipid groups titration.

These results support the previous hypothesis that the lipids are functionally involved in E protein ion channel activity, forming a protein-lipid pore, a novel concept for CoV E protein ion channel entity.

[1] DeDiego, M.L., et al 2008. Virology 376, 379-389.
[2] Verdiá-Báguena C., et al. 2012. Virology. 432: 485-494.

3250-Pos Board B405

Electrophysiology of Concatameric Pannexin 1 Channels Reveals the Stoichiometry of C-Terminal Autoinhibition

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Pannexin 1 (PANX1) is a non-selective ion channel that mediates the uptake of cyanine dyes and release of nucleotides and other metabolites. PANX1 activation and ATP release/dye uptake are regulated by diverse stimuli, including physicochemical factors (e.g., stretch, K^+ ions) and signaling by various G protein-coupled and ionotropic receptors. We recently described a unique regulatory mechanism for the release of ATP from apoptotic cells: PANX1 is activated by caspase cleavage of a C-terminal autoinhibitory domain. Current evidence suggests that PANX1 channels are hexameric, and cleavage-resistant subunits can interfere with caspase-dependent activation in a dominant-negative fashion. To explore the subunit stoichiometry required for C-terminal autoinhibition, we engineered concatameric PANX1 constructs

with different (from 0 to 6) combinations of truncated and full-length sequences. Formation of concatemers was assessed by the use of single-molecule photobleaching and protein cross-linking. Whole-cell recordings from concatenated PANX1 constructs suggest that at least four intact C-termini are required to inhibit channel activity. In addition, as the number of intact C-termini increased, there was a progressive decrease in single channel conductance, suggesting that individual C-termini may act within the multimeric channel to inhibit channel conductance. These results provide further mechanistic insights into the regulation of PANX1 channels by the C-terminal autoinhibitory domains.

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Structural Basis of Pannexin Activation

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Pannexin 1 (Panx1) is a member of a family of large-pore ion channels distantly related to invertebrate gap junction channels, the innexins. Activation of Panx1 occurs under a variety of physiological processes, but the molecular mechanism of such activation has not in general been clearly established. Recently it was shown that Panx1 could be activated by direct cleavage near the c-terminus by caspase-3. This occurs during apoptosis and leads to the release of large intracellular molecules such as ATP, which act as ''find-me'' signals enabling nearby phagocytes to quickly clear dying cells. The C-terminal domain peptide seems to function as a tethered pore blocker and is thought to have a specific interaction with structural elements within the pore to inhibit the channel. However, the basis for this structural interaction is poorly understood. Here we dissect those interactions using progressive alanine substitutions and long alanine repeats within the region distal to the caspase cleavage site. Our results suggest that any specific interaction between the pore and the C-terminus is minimal since replacement of the amino acid residues in the region adjacent to the caspase cleavage site with alanines does not produce a constitutively open channel. The most important feature of the C-terminal inhibitory peptide appears instead to simply be its length. We therefore propose that the c-terminal peptides loop back into the pore to reach a constricted region, thereby blocking the pore by sterically interfering with ion permeation. Peptides not long enough to reach this constriction within the pore are consequently unable to block the channel while longer peptides are able to block the pore in a promiscuous fashion.

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Pseudomonas Aerginosa Outer Membrane Carboxylate Channels Examined at the Single-Molecule Level Reveals Conserved Selectivity within each Subfamily

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Pseudomonas aerginosa is a Gram-negative bacterium, which employs unique outer membrane proteins for the uptake of small, water-soluble molecules. Here, we explore the two subfamilies of the outer membrane carboxylate channels, OccK and OccD, using single-molecule electrophysiology to probe the specific signature of each of the members of these two families. The seven member OccK family displays a broad range of unitary conductance values which includes low (~40−100 pS) and medium (~100−380 pS) conductance. These values are broader than what was expected from structural studies alone. It was also found that the OccK subfamily displays conserved anion selectivity, coinciding with the location of a net pool of positive charges within the channels' constriction sites. Single-channel activities of 6 members of the OccD subfamily also displayed a broad range of unitary conductance values between 20 and 670 pS. Single-channel electrophysiology studies indicated that the OccD subfamily members are all cation selective. Together these findings lead to a better understanding of the diversity within the outer membrane carboxylate channels of *Pseudomonas aerginosa*.

3253-Pos Board B408

A Simulation Approach to Molecular Transport in Bacterial Reaction Chambers

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Bacterial microcompartments (BMCs) are capsid-like assemblies that serve as simple protein-based metabolic organelles in bacterial cells. BMCs consist of a few thousand shell proteins that encapsulate specific enzymes, thus sequestering and/or enhancing certain metabolic pathways that often involve toxic intermediates. BMCs represent promising targets for rational modification in bionanotechnology. Crystallographic structures of some BMC proteins suggest that dynamic pores may be present within shell assemblies, providing the means for the controlled transport of substrates, products, and cofactors in the absence of a selectively permeable membrane. However, experimental evidence regarding BMC molecular transport remains inconclusive. In this study, all-atom, explicitly solvated molecular dynamics simulations have been used to generate microsecond-timescale sampling of molecular transport through several homohexameric shell assemblies from the archetypal BMC, the carboxysome. The carboxysome is specialized for carbon fixation in cyanobacterial cells, converting atmospheric carbon dioxide into organic compounds via the encapsulated enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO). It has been hypothesized that the shell may be porous to bicarbonate which is sequestered within the carboxysome following conversion to carbon dioxide, whilst oxygen may be excluded as it is known to inhibit the fixation process by RuBisCO. Molecular simulations have been used to characterize the permeability properties of carboxysome oligomers to water, salt, oxygen, carbon dioxide, and bicarbonate. Probability maps and corresponding potentials of mean force for selective transport of metabolic intermediates have been derived, and related to the size, electrostatics, and hydrogen-bonding characteristics of carboxysome capsid subunits. Moreover, conformationally dynamic regions of the BMC subunits were observed to partially occlude the pores, suggesting the possibility of gated transport. Overall, our observations provide insight into the molecular mechanisms of microcompartment transport, with fundamental implications for biological protein assemblies.

3254-Pos Board B409

An Unusual Aquaporin-Like Metalloid Boric Acid Channel in *Arabidopsis*

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The Major Instrinsic Protein/aquaporin superfamily is an ancient family with a conserved protein fold that forms a channel that mediates the bidirectional transport of water and uncharged solutes. The evolution of land plants was accompanied by a major diversification of the MIP gene family that resulted in the acquisition of new transport functions. Among these divergent plant specific MIP subfamilies are the ''nodulin 26 intrinsic proteins'' (NIPs), based on structural and functional homology to soybean nodulin 26. NIPs are divided into three subfamilies (NIP I, II and III) which show low to absent water permeability but have acquired the ability to transport a variety of uncharged solutes ranging from glycerol to boric acid to protonated lactic acid. In the present work we characterize the transport and gating properties of an unusual NIP II protein from *Arabidopsis* pollen microspores, AtNIP7;1 which is related to the boric acid channels AtNIP6;1 and 5;1, necessary for the uptake of this critical micronutrient. Functional characterization of NIP7;1 shows that unlike NIP5;1 and NIP6;1 which form constitutive boric acid channels, the intrinsic boric acid transport activity of NIP7;1 is extremely low. Molecular modeling suggests that a conserved tyrosine residue (Tyr 81) located in the transport pore stabilizes a closed conformation of the pore. Molecular dynamics simulation suggests that the closed conformation is stabilized by hydrogen bonding between the Tyr81 hydroxyl group and Arg 220 of the canonical ''aromatic-arginine'' selectivity filter. Since boric acid is both essential nutrient as well as a toxic compound at high concentrations, it is proposed that Tyr 81 modulates transport and provides an additional level of regulation of uptake of boric acid in male gametophyte development.

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Catsper Channel Organizes and Regulates Calcium Signaling Molecules in Spermatozoa

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Ejaculated mammalian spermatozoa gain the capacity to fertilize the egg within the female reproductive tract. Called capacitation, these changes to sperm include alterations in motility, membrane composition, and widespread protein tyrosine phosphorylation. Hyperactivated motility, the amplified, asymmetric movement of the tail, requires the sperm tail-specific Ca^{2+} channel, Cationic channel of sperm (CatSper). Calcium-mediated intraflagellar signaling is poorly understood, in part because flagellar diameters are less than 1 μm, preventing the resolution of specific molecular structures by conventional fluorescence microscopy. Here, we employed three-dimensional stochastic optical reconstruction microscopy (STORM) to determine how CatSper channel complex and other Ca^{2+} signaling molecules are organized. We find that the CatSper channel forms four linear nanodomains with calcium signaling molecules near the plasma membrane along the flagella. Strikingly, lack of the CatSper channel results in delocalization of Ca^{2+} signaling molecules. The spatial organization of protein components in CatSper signaling provides insights into Ca^{2+} signal transduction in regulation of sperm motility.