Our findings show that Nav1.6 SI can be severely altered by mutations in DII and DIII S6 segments, thereby confirming the importance of these regions to SI. N1455A induces loss of Nav1.6 during prolonged high frequency stimulation, which will allow us to test for the specific role of Nav1.6 vs. Nav1.7 in action potential (AP) generation: when transfected into DRGs, N1455A may attenuate prolonged high-frequency firing, whereas the threshold (thought to be set by Nav1.7) may remain unaffected. With this mutations we produced a useful molecular tool and approach for elucidating the role and influence of Nav1.6 SI on firing patterns in sensory neurons.

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Ionic Channels in Human Stem Cells Derived from Pulp of Deciduous Teeth

Antonio C. Cassola, Estela M. Cruvinel, C.P. Koiffman.
Sao Paulo University, São Paulo, Brazil.

Pulp of deciduous teeth contains mesenchymal stem cells originated from neural crest. These cells can be differentiated into several phenotypes (neuronal cells, adipocytes and odontoblasts). Functional expression of voltage-dependent channels (Na^+ and K^+) by these cells was investigated with special interest on the role of channels for the phenotype and differentiation.

Cells were obtained from deciduous teeth of children (5 to 8 year's old, protocol approved by local ethical committee). Harvested cells were maintained in DMEM:F12 medium, supplemented with fetal bovine serum. Currents were recorded in voltage clamp mode of patch-clamp technique, whole cell configuration. Amplitude and kinetics of currents induced by depolarizing pulses were analyzed. Expression of genes coding for channel proteins were identified by amplifying cDNAs, obtained by RT-PCR, with specific primers.

In the cell population half showed current through Nav, TTX-sensitive isoforms. The most conspicuous gene expressed was SCN1A (Nav1.1). Less but significant was the expression of SCN3A (Nav1.3) and SCN5A (Nav1.5). The expression of Kv is more variable in cell population. In some cells Kv channels activate rapidly with noisy currents. Probably currents pass through KCa. In fact, the expression of the gene MaxiK is strong. Some cells show K^+ current with fast activation and inactivation, resembling transient outward current. cDNA for isoform Kv4.3 was unequivocally detected.

Although functional channels were not detected, RNA transcript of gene HCN2 was found.

Regarding ion channels mesenchymal cells show phenotypic variability. The notorious regularity is the expression of Nav, associated to action potential firing. These cells seem committed to neuronal differentiation.

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Computational Insights into Atomistic Details of Na^+ Versus Ca^{2+} Discrimination in Sodium Channel Na_vAb

Song Ke, Anna Stary-Weinzinger.
University of Vienna, Vienna, Austria.

Voltage-gated Na^+ (Na_V) are required for rapid signal propagation in cardiac, muscle and nerve cells. How Na_V channels distinguish between sodium and calcium is an unresolved question. Selectivity is assumed to involve subtle mechanisms, since both ions have nearly identical size.

Interestingly, the selectivity filter of bacterial Na_V channels is expected to be quite similar with the filter of voltage gated calcium channels, since both contain a highly conserved selectivity filter signature sequence (TxExW). Despite this, bacterial Na_V channels are selective for sodium with permeability ratios P_{Ca}/P_{Na} of ~0.08-0.15 (Shaya et al. 2011).

To investigate how bacterial Na_VAb channels (Payandeh et al. 2011) discriminate between Ca^{2+} and Na^+, we performed molecular dynamics simulations. Firstly, double bilayer simulations with varying charge gradients and 200mM NaCl concentration were performed. As expected, rapid Na^+ influx is observed. Simulations with 200mM $CaCl_2$ revealed that Ca^{2+} ions can move into the selectivity filter, but not into the cavity, consequently no ion flux was observed. Additionally, calcium ions induced conformational changes of the glutamate side chain, which is in contrast to NaCl simulations, where the filter remained unchanged. To get detailed insights into this surprising behavior, we performed single-ion potential of mean force (PMF) calculations with Na^+ and Ca^{2+}.

PMF simulations revealed that there is no energy barrier higher than 2.1 kcal/mol for a Na^+ ion, suggesting that it can easily pass the filter. In contrast, Ca^{2+} encounters an energy barrier of approximately 13.8 kcal/mol, preventing calcium ions from moving into the cavity.

Results of our simulations are consistent with a recent X-ray structure on the Na_VRh channel (Zhang et al. 2012), showing a Ca^{2+} ion bound to the same site of the selectivity filter as seen in this study for Na_VAb.

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On Conduction and Permeation in a Bacterial Sodium Channel

Carmen Domene[1], Simone Furini[2].
[1]University of Oxford, Oxford, United Kingdom, [2]University of Siena, Siena, Italy.

Voltage-gated Na^+-channels are transmembrane proteins that are responsible for the fast depolarizing phase of the action potential in nerve and muscular cells. They are essential for the rapid depolarization of nerve and muscle1, and are important drug targets2. Selective permeability of Na^+ over Ca^{2+} or K^+ ions is essential for their biological function. An understanding of how these channels discriminate between different ion types and how ions permeate the pore is not well understood yet. The X-ray structure of the bacterial sodium channel NavAb has provided a new template for the study of sodium. An anionic coordination site was proposed to confer Na^+ selectivity through partial dehydration of Na^+ via its direct interaction with conserved glutamate side chains. Starting from the crystal structure and by combining molecular dynamics simulations and free-energy calculations a low-energy permeation pathway for Na^+ ion translocation through the selectivity filter NavAb is characterised. The picture that emerges is that of a pore preferentially occupied by two ions, which can switch between different configurations by crossing low energy-barriers. In contrast to K^+-channels, the movements of the ions appear to be weakly coupled in Na^+-channels. When the energy maps for Na^+ and K^+ ions are compared, a selective site is characterised in the narrowest region of the filter, where a hydrated Na^+ ion, and not a hydrated K^+ ion, is energetically stable.

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Selectivity between Sodium, Calcium and Potassium Ions in Bacterial Sodium and Calcium Channels

Ben Corry.
Australian National University, Acton, ACT, Australia.

The families of voltage gated sodium and calcium channels are responsible for the upstroke of action potentials and converting electrical signals into cellular responses. The ability for these channels to be able to transport only the desired ion species across the membrane is critical to their function. The recent publication of a structure of a bacterial voltage gated sodium channel has opened the door for the mechanisms of selectivity in both these channel families to be investigated.

Using molecular dynamics simulations and free energy calculations we are able to show how a range of bacterial sodium and calcium selective channels are able to differentiate between ion species. The selectivity of NavAB and NaChBac for sodium over potassium is shown to result from the inability of potassium to fit through the narrowest portion of the channel with the preferred solvation geometry. In contrast, rejection of calcium happens slightly further into the channel where the square planar coordination sites more aptly fit sodium ions. The gradual mutation of NavAB into the calcium selective channel CaChBac progressively makes the high field strength sites more favourable for calcium to the point where it out competes sodium ions.

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Exceptionally Conserved Asparagines in the Pore-Lining Helices of Calcium and Sodium Channels Stabilize the Open State through Interdomain H-Bonds

Denis B. Tikhonov[1], Iva Bruhova[2], Daniel P. Garden[2], **Boris S. Zhorov**[1,2].
[1]Sechenov Institure, RAS, St. Petersburg, Russian Federation, [2]McMaster University, Hamilton, ON, Canada.

The cytoplasmic halves of the pore-lining helices in voltage-gated sodium and calcium channels contain exceptionally conserved asparagine residues in each of the four repeat domains. The exceptional conservation implies important roles of the asparagines, which are unknown. Previous studies demonstrated that mutations of the asparagines affect activation and slow inactivation of the channels, as well as action of pore-binding drugs, including local anaesthetics and mibefradil. In the absence of open-channel structures, underlying mechanisms are unclear. Here we modeled the open, closed, and inactivated conformations of Cav1.2 and Nav1.5 channels using available x-ray structures of potassium and sodium channels as templates. The energy of each channel state was optimized by the Monte Carlo-minimization protocol. The asparagines do not face the pore lumen in any of the modeled states. In the closed channel models, the asparagines occur in hydrophobic environments that would bring a destabilizing contribution to the closed state energy. In the open-channel models, the asparagine residue in each domain forms an interdomain H-bond with a polar residue, which is typically nine positions downstream from the conserved asparagine in the preceding domain. The H-bonds are