

113-Plat**Calmodulin Binding to a Novel Site in the AB Module of Kv7.2 Subunits Regulates Surface Expression**

Ganeko Bernardo-Seisdedos¹, Juncal Fernandez-Orth¹, Carolina Gomis-Perez¹, Alessandro Alaimo¹, Araitz Alberdi¹, Covadonga Malo¹, Pilar Areso², Alvaro Villarroel¹.

¹CSIC, Leioa, Spain, ²UPV/EHU, Leioa, Spain.

Calmodulin (CaM) binding to the AB module underlie multiple mechanisms governing the function of Kv7.2 subunits, which are the main component of the non-inactivating K⁺ M-current, a key controller of neuronal excitability. Simultaneous binding to helix A and B is crucial for trafficking to the plasma membrane. We have identified a novel CaM-binding site located downstream of helix A, reminiscent of the TW motif of CaM-gated SK2 K⁺ channels, with W360 as pivotal for surface expression. In the context of the known CaM-binding modes and previous studies, our data suggest a model for traffic control that involves alternative CaM docking to either helix B, the novel TW motif, or the IQ-site of helix A.

114-Plat**A Point Mutation Causing Episodic Ataxia Reveals Functional Link between Voltage Sensor and Selectivity Filter in Shaker Kv Channels**

Dimitri Petitjean^{1,2}, **Rikard Blunck^{1,2}**.

¹Departments of Physics and of Physiology, Université de Montréal, Montréal, QC, Canada, ²GÉPROM, (Membrane Protein Research Group), QC, Canada.

Episodic ataxia (EA) constitutes itself as sporadic episodes of vertigo, migraine and ataxia and is occasionally associated with epilepsy or hemiplegic migraine. EA type 1 is caused by mutations in KCNA1. We investigated the biophysical implications of one of these mutations, F244C, in the drosophila analogue the Shaker Kv channel (numbering according to Shaker). In spite of being located in the first transmembrane helix (S1), the mutation had drastic effects on the gating currents. The gating charge-voltage relation (QV) was shifted to more depolarized potentials. Although other EA1 mutations had been linked to electromechanical coupling, this was not the case for F244, as also the conductance-voltage relations of different amino acid replacements were proportionally shifted to more depolarized potentials. Instead, we found a stabilization of the second arginine in S4 in the intermediate state of the voltage sensor dependent on the hydrophobicity of the replacement residue.

One striking feature of the F244C mutant was that it led to drastic development of leak in the W434F background. This leak current passed through the main ion conducting pore, and we were able to link it to reduced C-type inactivation. We established that the C-type inactivation of the neighboring subunit was effected. F244 therefore directly links the voltage sensor movement of one subunit with the selectivity filter of its neighboring one.

This work is supported by grants of the Canadian Institutes of Health Research and the Canada Research Chairs.

115-Plat**Evolutionary Origins of the Shaker Family of Voltage-Gated Potassium Channels**

Xiaofan Li¹, Hansi Liu¹, Sarah A. Rhodes¹, Liana Trigg¹, Fortunay H. Diatta¹, Jessica K. Sassic¹, David K. Simmons², Mark Q. Martindale², Timothy Jegla¹.

¹Biology, The Pennsylvania State University, University Park, PA, USA,

²The Whitney Marine Laboratory for Marine Science, University of Florida, St. Augustine, FL, USA.

The Shaker family of voltage-gated K⁺ channels in bilaterians consists of four closely related gene subfamilies (Shaker, Shab, Shaw and Shal) that regulate neuronal excitability in diverse ways. We examined the evolutionary origins of the gene family by characterizing Shaker channels in early branching metazoan lineages. Shaker family channels can be definitively identified by the combination of a voltage-dependent potassium channel core and a cytosolic domain (T1) that mediates subfamily-specific tetrameric assembly. The Shaker, Shab, Shaw and Shal subfamilies are present in cnidarians, a close sister group to the bilaterians. We show that all four subfamilies are highly conserved on a functional level in the sea anemone *Nematostella vectensis*, an emerging cnidarian model organism. The *Nematostella* Shaker, Shal and Shaw subfamilies include cnidarian-specific expansions of regulatory subunits that extend functional diversity through the formation of heteromeric channels. We were unable to identify Shaker family genes in choanoflagellates or sponges, but found over 40 Shaker family genes in a ctenophore (*Mnemiopsis leidyi*). Ctenophores are the earliest branching metazoans with a nervous system. Phylogenetic analysis indicates that the ctenophore Shaker family expanded independently and diverged prior to emergence of the Shaker, Shab, Shal and Shaw subfamilies. The phylogeny and functional analysis support Shaker as the oldest of these subfamilies. Two *Mnemiopsis* Shaker family subunits heteromerize with *Nematostella* and mouse Shaker subfamily channels, but not with Shab, Shaw or Shal. Thus the subunit

interface of Shaker subfamily may retain ancestral features. Our results indicate that the Shaker family is metazoan-specific and was present by the time neurons evolved. However, the Shaker, Shab, Shal and Shaw subfamilies evolved in the cnidarian/bilaterian lineage long after the evolution of the first nervous systems.

116-Plat**Alternative Splicing Modulates Kv Channel Clustering through a Molecular ‘Ball and Chain’ Mechanism**

Nitzan Zandany, Shir Marciano, Elhanan Magidovich, Rinat Yehezkel, Tzilhav Shem-Ad, Limor Lewin, Uri Abdu, Irit Orr, **Ofer Yifrach**.

Life Sciences, Ben Gurion University of the Negev, Beer sheva, Israel.

Ion channel clustering at the post-synaptic density (PSD) serves a fundamental role in action potential transmission. In voltage-activated potassium channels (Kv), this process is mediated by interaction of the C-terminal tail with scaffold proteins by a currently unclear mechanism. Here, we show that interaction between the prototypical Shaker Kv channel and the PSD-95 scaffold protein is entropy-controlled and modulated by the length of the intrinsically disordered channel tail. We further show that the Kv channel tail functions as entropic clock that times scaffold protein binding. Based on these observations, we propose a ‘ball and chain’ mechanism to explain C-terminal-based Kv channel binding to scaffold proteins, analogous to the classical N-type mechanism that describes channel fast inactivation. The physiological relevance of this mechanism is demonstrated by showing that alternative splicing in the Shaker Kv channel gene, producing channel variants with distinct C-terminal tail lengths, exhibit distinct scaffold protein-mediated channel cell surface expression and clustering patterns that correlate with differences in affinity of the variants to PSD-95. We suggest that modulating channel clustering by specific spatial-temporal variant targeting serves a fundamental role in nervous system development and tuning.

117-Plat**β-2 and γ-1 Auxiliary Subunits Coassemble in the Same BK Channel and Independently Contribute to Regulation of Channel Gating**

Vivian M. Gonzalez-Perez, Xiao-Ming Xia, Christopher J. Lingle.

Anesthesiology, Washington University in St. Louis, St. Louis, MO, USA.

Large conductance, Ca²⁺-and-voltage activated K⁺ (BK) channels are oligomeric proteins whose minimal functional unit is a homotetramer of the pore-forming α subunit. The α subunit is almost ubiquitously expressed in mammalian tissues, but native BK channel complexes can also contain associated auxiliary subunits which are tissue-specifically expressed. Members of two structurally distinct families of auxiliary subunits, β1-β4 and γ1-γ3, have each been shown to separately assemble with BK-α subunit. Each auxiliary subunit defines important gating properties of the BK channel complex as well as its pharmacology. However, the possibility of coassembly of the two types of auxiliary subunits in the same BK channel has not been closely examined. The only antecedent reports that overexpression of β1 subunit precludes the ability of γ1 to affect BK gating. Here, using single channel and macroscopic patch-clamp recordings we show that β2 and γ1 can coassemble in the same channel. When α, β2 and γ1 subunits are coexpressed at typical RNA ratios, the resulting BK-current shows a complete inactivation, indicating the presence of β2 in all channels, but also exhibits a gating shift consistent with the presence of γ1 in all channels. The effects produced by β2 and γ1 subunits on BK channel gating are approximately additive, consistent with independent mechanistic effects. Furthermore, coassembly of both subunits in the same channel produces a constitutive inactivation of BK current at physiological conditions even at 0 [Ca²⁺]. This possibility that β and γ subunits may also coassemble in the same BK channels in native tissues adds significantly to the potential functional diversity of BK channels.

This work was supported by National Institutes of Health Grant GM-081748 (to C.J.L.) and National Research Service Award GM103138 (to V.G.-P.).

Platform: Cytoskeletal Mechanics, Dynamics, Motility, and Myosins**118-Plat****Site-Specific Cation Release Drives Actin Filament Severing by Vertebrate Cofilin**

Hyeran Kang¹, Michael J. Bradley¹, Wenxiang Cao¹, Kaifeng Zhou¹, Elena E. Grintsevich², Alphonse Michelot³, Emil Reisler², Charles V. Sindelar¹, Mark Hochstrasser¹, Enrique M. De La Cruz¹.

¹Molecular Biophysics and Biochemistry, Yale University, New Haven, CT, USA, ²Chemistry and Biochemistry, UCLA, Los Angeles, CA, USA,

³Physics of the Cytoskeleton and Morphogenesis Group, iRTSV, LPCV/CNRS/CEA/INRA/UJF, Grenoble, France.

Actin polymerization powers the directed motility of eukaryotic cells. Sustained motility requires rapid filament turnover and subunit recycling. The