function of ion channels formed by two antimicrobial peptides, gA and Alm. We find that GS stabilizes both gA channels and the higher conductance states in Alm channels, indicating that GS increases bilayer elasticity at concentrations below those at which it by itself causes breakdown of the bilayer barrier properties. The presence of one membrane protein may affect the stability and function of another membrane protein through changes in the physical properties of the host bilayer, a finding likely to be relevant for the function of biological membranes and possibly for the mode of action of antimicrobial peptides.

22-Plat New Insights into Red Blood Cell Membrane Dynamics

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The dynamical behavior of the red blood cell (RBC) and the relationship of membrane fluctuations with viscoelasticity are still open questions. We report both forced and spontaneous motions of microbeads tightly bound to the RBC membrane. To assess the nature of forces driving membrane dynamics we studied the effects of temperature and ATP depletion. We found that RBC stiffness was nearly frequency independent and that spontaneous bead motions were sub-diffusive or caged for almost all time lags experimentally accessible (~ 0.05–50 sec). We found that from room temperature to febrile 41°C cell stiffness approximately doubled. Consistent with this finding, increased temperature slowed down the spontaneous motion of the beads. ATP depletion led to a much larger stiffening response, approximately an order of magnitude. These measurements indicate that ATP associated effects dominate thermal ones. Moreover, our data relate spontaneous fluctuations directly to dissipation seen in driven motion and allows us to investigate the extent to which the fluctuation-dissipation theorem holds in RBCs.

Platform B: Voltage-gated Ca Channels

23-Plat Calmodulin-dependent Gating of Calcium Channels in the Absence of β Subunits

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It is generally accepted that, to generate calcium currents in response to depolarization, $Ca_v1.2$ calcium channels require association of the poreforming α1C subunit with the β and α2δ accessory subunits. At least one calmodulin molecule (CaM) is tethered to the C-terminal α1C-LA/IQ region and mediates Ca^{2+}-dependent inactivation (CDI) of the channel. β subunits are stably associated with the α1C-AID site of the cytoplasmic linker between internal repeats I and II and also interact dynamically, in a Ca^{2+}-dependent manner, with the α1C-IQ motif. Here we describe a surprising discovery that CaM supports calcium channel voltage gating on transient coexpression with α1C/α2δ in COS1 cells in the absence of β subunits. The β-free CaM-activated α1C/α2δ channels exhibited CDI. Real time PCR with primers complementary to monkey β subunits did not reveal an induction of endogenous β subunits in response to overexpression of CaM indicating that calcium channel-free environment of the COS1 cell expression system was not compromised by CaM. Similar to CaM, transient expression of the calcium-insensitive CaM_{1234} mutant activated β-free $Ca_v1.2$ channels and stimulated the plasma membrane targeting of α1C/α2δ complexes, but did not support CDI. Our results suggest that there is a calcium-independent crosstalk between CaM and β subunits that, in the absence of β, allows for facilitation of the channel voltage gating via interaction with CaMs other than the regularly tethered one. This effect is altered or abolished by the α1C-β association. Thus, transient expression of CaM creates conditions when the channel gating, inactivation, CDI and plasma-membrane targeting occur in the absence of β.

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24-Plat

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25-Plat Origin Of The Voltage Dependence Of G Protein Regulation Of P/Q-type Ca^{2+} Channels

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G protein (Gβγ)-mediated voltage-dependent inhibition of N- and P/Q-type Ca^{2+} channels contributes to presynaptic inhibition and short-term synaptic plasticity. The voltage dependence arises from the dissociation of Gβγ from the inhibited channels, and it is this property that permits high frequency action potential firing to relieve the inhibition of synaptic N- and P/Q-type Ca^{2+} channels by G-protein coupled receptors. The molecular and biophysical mechanisms underlying Gβγ unbinding remain largely unclear. We have investigated the structural elements and conformational changes that produce the voltage dependence. We found that voltage-dependent Gβγ inhibition required the calcium channel β subunit ($Ca_v\beta$) and a rigid α-helical structure between the AID, the primary $Ca_v\beta$ docking site on the channel α_1 subunit, and the pore-lining IS6 segment. Gβγ inhibition of P/Q-type channels was reconstituted in inside-out membrane patches from *Xenopus* oocytes by applying purified Gβγ directly to the cytoplasmic side of the channels. Channels devoid of Caβ (produced by removing a mutant $Ca_v\beta$ with a reduced affinity for the AID) or containing a WT $Ca_v\beta$ but bearing a helix-disrupting insertion between the AID and IS6 were still inhibited by Gβγ, but without any voltage dependence. Furthermore, a truncated $Ca_v\beta$ containing only the AID-binding guanylate kinase (GK) domain could fully confer the voltage dependence. These results suggest that depolarization-triggered movement of IS6, coupled to the subsequent conformational change of the Gβγ-binding pocket through a rigid a-helix induced partly by

the $Ca_v\beta$ GK domain, causes the dissociation of $G\beta\gamma$ and is fundamental to voltage-dependent $G\beta\gamma$ inhibition.

26-Plat Atypical Properties of a Conventional Ca2+ Channel Beta Subunit from the Platyhelminth Schistosoma mansoni

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The function of voltage-gated calcium (Cav) channels greatly depends on coupling to cytoplasmic accessory beta subunits, which not only promote surface expression, but also modulate gating and kinetic properties of the pore-forming alpha1 subunits. Schistosomes, parasitic platyhelminths that cause schistosomiasis, express two beta subunit subtypes: a structurally conventional beta subunit and a variant beta subunit with unusual functional properties. We have previously characterized the functional properties of the variant Cav beta subunit. Here we focus on the modulatory phenotype of the conventional Cav beta subunit (SmCav beta) using the human alpha1E channel (Cav2.3) stably expressed in HEK-293 cells and the whole-cell patch-clamp technique. As expected, the conventional Cav beta subunit dramatically increases Cav2.3 currents, slows macroscopic inactivation and shifts steady state inactivation in the hyperpolarizing direction. However, we found that currents produced by Cav2.3 in the presence of SmCav beta run down to approximately 75% of their initial amplitudes within two minutes of establishing the whole-cell configuration. This suppressive effect was independent of Ca2+. Further exploration revealed that

(i) a physiological form of ATP (Mg-ATP) was required for this run-down,
(ii) SmCav beta potentiates the suppressive effect of Mg2+ on Cav2.3 channels, and
(iii) SmCav beta confers Na+ sensitivity to the Cav2.3/SmCav beta complex.

These data provide insights into novel mechanisms employed by platyhelminth Cav beta subunits to modulate voltage-gated calcium currents.

27-Plat Spatial Probing of Skeletal Muscle Calcium Channel Beta1a Subunit Using Bimolecular Fluroescence Complementation

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In this study, we employed the technique of Bimolecular Fluorescence Complementation (BiFC) to probe the spatial characteristics of the β1a subunit of the skeletal muscle dihydropyridine receptor (DHPR). Yellow Fluorescent Protein (YFP) was cleaved into separate entities of amino acids 1–158 (5′SplitY) and 159–238 (3′SplitY). When expressed separately, neither construct fluoresces yellow upon excitation with a 514 nm Argon laser. We tagged the DHPR β1a subunit with 5′SplitY on the N-terminus and/or 3′SplitY on the C-terminus. Coexpression of β1a-3′SplitY and the complementary, free 5′SplitY in β 1KO myotubes produced yellow fluorescence, robust L-type calcium currents, and normal intramembrane charge movement. When the constructs 5′SplitY-β1a and β1a-3′SplitY were coexpressed in the same cell no yellow fluorescence was observed. Alternatively, the construct 5′SplitY-β1a-3′SplitY fluoresces brightly and reconstitutes normal calcium currents and excitation-contraction coupling in β1KO myotubes. These findings support previous studies that suggest

1. the β1a C-terminus is accessible to a large molecular probe when targeted to normal triad junctions,
2. the N-and C-termini of neighboring β1a in a DHPR tetrad arrangement are not close to each other (>10nm), and
3. the N-and C-termini of a single β1a subunit fold close to each other (<10nm).

These experiments suggest the BiFC technique may be a useful tool in determining potential protein-protein interactions between the skeletal muscle calcium channels DHPR and RyR1.

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28-Plat Enhancement Of Voltage-dependent Inactivation Of The Cav1.2 Channel By Its Proteolytically Processed Distal C-terminus

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L-type Ca current through $Ca_V1.2$ channels initiates excitation-contraction coupling in the heart. Voltage-dependent inactivation (VDI) is faster for L-type Ca current in myocytes than for $Ca_V1.2$ expressed in tsA-201 cells. We investigated molecular elements that could be responsible for this difference. In whole-cell patch clamp experiments, sodium was used as the charge carrier to eliminate the effects of permeant divalent ions on inactivation of the Ca current. The fraction of peak current remaining at the end of a 1000-ms depolarization to 0 mV (r_{1000}) was used to quantify inactivation. Because the C-terminus of $Ca_V1.2$ is proteolytically processed in ventricular tissues and has autoinhibitory effects on $Ca_V1.2$ channels, we tested the impact of truncating $Ca_V1.2$ at position 1821 ($Ca_V1.2\Delta1821$). There was no significant difference in VDI between $Ca_V1.2\Delta1821$ and full-length $Ca_V1.2$. In contrast, when $distal_{1822–2171}$ was expressed together with $Ca_V1.2\Delta1821$, inactivation was more pronounced than that of $Ca_V1.2\Delta1821$ alone. r_{1000} was 0.54 ± 0.03 (n=10) for $Ca_V1.2\Delta1821$ versus 0.28 ± 0.03 (n=21) for $Ca_V1.2\Delta1821$ with $distal_{1822–2171}$ ($p<0.001$). We also investigated the impact of different $Ca_V\beta$-subunits on VDI of $Ca_V1.2$. $Ca_V\beta_{2b}$ slightly increased the inactivation of $Ca_V1.2$ compared to $Ca_V\beta_{1b}$. r_{1000} for $Ca_V\beta_{2b}$ was 0.54 ± 0.03 (n=9) versus 0.69 ± 0.05 (n=7) for $Ca_V\beta_{1b}$ ($p<0.05$). Our results show that noncovalent

association of the distal C-terminus significantly enhanced VDI of $Ca_V1.2$ channels and that the effects of different β-subunits on VDI were small relative to the distal C-terminus. These findings expand the functional repertoire assigned to the distal C-terminal domain and support an important role in regulating Ca influx via its control of VDI.

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29-Plat C-terminal Splicing Fine-tunes Typical Low-voltage Activation Of Cav1.3 L-type Calcium Channels

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Low-voltage activation of Cav1.3 L-type Ca2+ channels (LTCCs) controls excitability in sensory and central neurons as well sinoatrial node pacemaking. Cav1.3 mediated pace-making determines neuronal vulnerability of dopaminergic striatal neurons affected in Parkinsons' disease.

We have previously identified an intrinsic distal C-terminal modulator (CTM) that regulates voltage- and calcium-dependent gating of Cav1.4 LTCC. Given the high homology in the C-terminus we hypothesize that a short Cav1.3 (Cav1.3S) splice variant that lacks potential corresponding CTM would also be affected in its gating properties. We therefore expressed either the full length (Cav1.3L) or the short Cav1.3S splice form together with β3 and α2δ-1 in tsA-201 cells and determined their biophysical properties using whole-cell patch-clamp technique. Ca2+ currents through Cav1.3S activated at more negative voltages (V0.5act: Cav1.3S: −12.9±0.8mV, n=15; Cav1.3L: −0.8±0.8mV, n=16: $p<0.0001$), inactivated faster (fraction of peak current remaining after 250ms: Cav1.3S: 0.13±0.02, n=6; Cav1.3L: 0.46±0.05, n=5; $p<0.01$) and showed more significant CDI ($p<0.01$). Window current was shifted to more negative potentials. The short Cav1.3 splice variant is significantly expressed in human brain, retina and heart and several mouse brain regions, cochlea and eye as identified in qualitative and quantitative RT-PCR experiments. Moreover, we have identified the Cav1.3 CTM within the last 116 amino acids.

These experiments revealed a novel mechanism of channel modulation in Cav1.3 LTCCs enabling alternative splicing to tightly control channel gating. The absence of the CTM in short splice forms leads to Cav1.3 channels activating at even lower voltages than previously reported for Cav1.3L very likely to fine-tune Cav1.3 function at negative voltages as required for the modulation of neuronal firing behaviour and sinoatrial node pacemaking.

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30-Plat Amino-terminal CaM binding Site (*NSCaTE*) Specifies Contrasts in Spatial Ca^{2+} Selectivity of Ca^{2+}-dependent Inactivation (CDI) in Ca_V1 versus Ca_V2 Channel Clades

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Ca^{2+}-dependent regulation of Ca^{2+} channels is orchestrated by a single calmodulin (CaM), constitutively associated with the carboxy termini of Ca_V1-2 channels. Remarkably, Ca^{2+} binding to each lobe of CaM can trigger distinct forms of regulation on a host channel. Furthermore, whereas the C-lobe responds to the intense local Ca^{2+} signal generated by the home channel ('local Ca^{2+} selectivity'), the N-lobe somehow favors the weaker cumulative Ca^{2+} signal generated by multiple Ca^{2+} sources over larger spatial domains ('global Ca^{2+} selectivity). The latter selectivity is vital for spatially coordinated Ca^{2+} feedback, yet the malleability of this detection mode has been uncertain. Here, we show that such N-lobe selectivity can be transformed by a Ca^{2+}/CaM binding site (*NSCaTE*: N-Terminal Spatial Ca^{2+} Transforming Element), situated within the amino termini of certain channels. For Ca_V2 channels, *NSCaTE* is absent and N-lobe CDI exhibits a global selectivity. By contrast, in $Ca_V1.2$ and $Ca_V1.3$, where *NSCaTE* is naturally present, N-lobe CDI adopts a local selectivity. The transforming potential of *NSCaTE* is linked to Ca^{2+}/CaM binding, as deletions impacting binding revert N-lobe CDI to a global profile. Additionally, three *NSCaTE* residues are crucial for Ca^{2+}/CaM interaction, and perturbations of these reveal a tight correlation between Ca^{2+}/CaM binding and spatial selectivity. Finally, *NSCaTE* effects are transferable, as tethering *NSCaTE* to $Ca_V2.1$ and $Ca_V2.2$ channels changes their N-lobe CDI towards local selectivity. This effect of an amino terminal element is unprecedented, given the preponderance of known structural determinants for CaM regulation on channel carboxy termini. Indeed, the amino-terminal position of *NSCaTE* argues that Ca^{2+}/CaM can bridge the carboxy and amino termini of $Ca_V1.2$/$Ca_V1.3$ channels, expanding on a theme wherein CaM can crosslink separate parts of a single molecule.

Platform C: Structure Function of Membrane Transport

31-Plat How Subunit Coupling Produces the Rotary Motion In F_1-ATPase: Insights from Simulation

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F_oF_1-ATP synthase manufactures the energy "currency", ATP, of living cells. The soluble F_1 portion, called F_1-ATPase, can act as a rotary motor, with ATP binding, hydrolysis, and product release, inducing a torque on the gamma subunit. A coarse grained plastic network model has been used to show at a residue level of detail how the conformational changes of the catalytic beta subunits act on the