channels featuring a systematic swap of all possible permutations of intracellular loops/termini of α_{1C} into analogous positions in the β-independent, low-voltage-activated $Ca_V3.1$ channel α_{1G} subunit, and studied functional reconstitution of β-dependence using electrophysiological recordings in HEK 293 cells. Surprisingly, functional analyses of chimeras yielded a pattern consistent with α_{1C} I-II loop possessing a net ER export capability that was opposed by discrete ER retention signals in the other cytoplasmic domains. Alanine scanning mutagenesis identified a cluster of acidic residues responsible for the ER export function of the α_{1C} I-II loop. Reconstitution of β-dependent increase in current required at least four α_{1C} intracellular domains, with both the I-II loop and C-terminus being essential. The results support a new model of $Ca_V1.2$ trafficking where β binding to α_{1C} I-II loop causes a C-terminus-dependent rearrangement of intracellular domains that shifts the balance of power between export signals on the I-II loop and retention signals elsewhere on the protein.

2877-Plat
Molecular Determinants of Voltage-Gated Calcium Channel Inhibition by Gem
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High voltage-activated (HVA) Ca^{2+} channels, including L-, N- and P/Q-type channels, are potently inhibited by the Rem, Rem2, Rad, and Gem/Kir (RGK) family of small GTP-binding proteins. This inhibition was widely thought to depend on the direct association between RGK proteins and the β subunit ($Ca_v\beta$) of HVA Ca^{2+} channels, but we recently found that P/Q channel inhibition by Gem protein in inside-out membrane patches required $Ca_v\beta$ but not the Gem-$Ca_v\beta$ interaction, and that Gem coimmunoprecipitated with the P/Q channel α_1 subunit in a $Ca_v\beta$-independent manner. Thus, we proposed that direct interactions between Gem and the α_1 subunit ($Ca_v\alpha_1$) of HVA Ca^{2+} channels are critical for Gem inhibition. In this study, we investigate the molecular determinants on $Ca_v\alpha_1$ that are important for Gem inhibition. We construct chimeras between the α_1 subunit of P/Q channels and the Gem-insensitive low voltage-activated T-type channels, which do not bind or require $Ca_v\beta$. We find that grafting the α-interaction domain (AID), the high-affinity $Ca_v\beta$ binding site on $Ca_v\alpha_1$, or grafting the entire AID-containing I-II loop from P/Q channel into T-channels ($T_{PQ\ I\text{-}II\ loop}$), is sufficient for $Ca_v\beta$ binding and gating regulation but does not bestow RGK inhibition. However, adding the first two transmembrane segments (IIS1-IIS2) of the second homology repeat onto the $T_{PQ\ I\text{-}II\ loop}$ construct, produced a Gem-sensitive T-channel. This inhibition persists with non-interacting Gem and $Ca_v\beta$ mutants, indicating that the $Ca_v\beta$-Gem interaction is not necessary, just as in the case of Gem inhibition of P/Q channels. In complementary experiments, substituting only IIS1-IIS2 of P/Q channel α_1 with that of T-channel's severely attenuates RGK inhibition. This supports a paradigm in which Gem directly binds and inhibits $Ca_v\beta$-primed $Ca_v\alpha_1$ on the plasma membrane. We are currently investigating the role of other $Ca_v\alpha_1$ regions in Gem inhibition.

2878-Plat
Calmodulin Interferes with $Ca_v1.2$ C-Terminal Regulation of L-Type Channel Current
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The L-type Ca channel (CaV1.2) distal carboxyl-terminus (CCt) has multiple functions. CCt inhibits L-type channel current, and is a mobile element that translocates to the nucleus where it regulates CaV1.2 transcription. CCt interacts with CaV1.2 in a similar domain as calmodulin (CaM). The purpose of this study is test the hypothesis that CaM and CCt compete for functional interaction with CaV1.2. CaV1.2 calcium current (ICa,L) and barium current (IBa,L) were recorded from HEK 293 cells transfected with CaV1.2 + CaVbeta2a. This background was compared to cells additionally transfected with CaM and/or CCt. The CaV1.2 expressed was deleted at position 1733 or 1801 (numbering based on rabbit sequence), and CCt corresponded to amino acids 1821-2171. CCt co-expression significantly reduced IBa,L, but not ICa,L. CCt inhibition of ICa,L is reversed by exogenous CaM co-expression, but not by calcium binding deficient apo-CaM. Examination of the peak I(V) curves suggests that midpoint of activation was not affected. Mouse ventricular cardiomyocytes transfected with CCt also showed a reduction in Cav1.2 IBa,L, but no reduction in ICa,L. Exogenous CaM co-expression also relieved CCt auto-inhibiton in mouse ventricular cardiomyocytes. We conclude that CCt attenuation of current occurs only with Ba, and is consistent with a Ca alleviation of CCt block. Thus, CaM and Ca functionally compete to limit CCt auto-inhibition of CaV1.2 current.

2879-Plat
Molecular Events Beyond apoCAM Preassociation in the CAM Regulation of Cav1.3 Channels
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Ca_V channel regulation by calmodulin (CaM) is a central prototype for ion-channel modulation. Despite long study, relatively little is known mechanistically, beyond the initial preassociation of Ca^{2+}-free CaM (apoCaM) with an IQ domain on the carboxy-terminus of channels. Most studies have focused on the IQ domain and immediate upstream 'preIQ' regions, despite hints that further upstream elements in the carboxy-terminus could be important. Accordingly, we here undertake alanine scanning mutagenesis of the entire carboxy-terminus upstream of the IQ domain (the proximal CI-region, PCI). For analysis, we chose $Ca_V1.3$ channels (highly homologous to classic $Ca_V1.2$), because they exhibit robust CaM-mediated inactivation (CDI), with particularly well-resolved profiles for both N- and C-lobe forms of inactivation. Several unexpected results were obtained. First, mutations throughout the preIQ domain left CDI essentially unchanged, at odds with functional hotspots in the homologous region of $Ca_V1.2$. Second, newly identified segments, situated upstream of the preIQ region, proved selectively critical for the C-lobe form of CDI. Specifically, we argue that the PCI region is the Ca^{2+}/CaM effector site for C-lobe CDI, as revealed by quantitative comparison of the effect of PCI mutations on CDI, to their effect on PCI binding with Ca^{2+}/CaM ('Ψ-analysis'). Third, we further exploit Ψ-analysis to extend and confirm that the Ca^{2+}/CaM effector site for the N-lobe form of CDI is structurally distinct, residing in the *NSCaTE* element of the channel amino terminus (*Nature* **451:**830). Finally, while the IQ-domain is a primary site for apoCaM preassociation, our scan surprisingly reveals that PCI harbors additional preassociation sites, especially important for the N-lobe of apoCaM. Overall, this alanine scan of the $Ca_V1.3$, together with that of the IQ domain (companion abstract), outlines the long-sought molecular events beyond the initial apoCaM preassociation with the channel.

Platform BF: Microtubular Motors

2880-Plat
Detailed Analysis of the Dynein Stepping Mechanism using Multicolor Tracking
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Cytoplasmic dynein is a motor protein that moves processively towards the microtubule minus end. To characterize dynein's step size at both limiting (5 μM) and saturating (1 mM) ATP concentrations, we have improved the temporal resolution of FIONA to 2 milliseconds and tracked the movement of single yeast cytoplasmic dynein motors, each labeled with one quantum dot. In contrast to kinesin, dynein's step size is highly variable, and backwards and sideways steps are frequently observed. As the tail domain takes ~8 nm steps, the heads advance by taking both short (~8 nm) and long (~16 nm) steps. These data indicate that the heads do not move in a strictly alternating manner, as is the case with kinesin and myosin. To further characterize how the dynein heads move relative to each other, we have tracked the movement of both heads simultaneously. To precisely measure the head-head separation vector, we have developed novel small quantum dots (10 nm diameter) that specifically attach to each dynein head. The fluorescent signals of the differently-colored quantum dots attached to dynein are then registered with 2 nm precision. Using this technique, we find the heads to be widely separated, mostly in the off-axis, indicating that the two heads walk along separate filaments. Our two-color stepping data also show that the dynein heads advance mostly by taking alternating steps, as is the case with kinesin. However, the heads may also move independently of each other, and one head may take multiple steps before its partner moves forward. Both the leading head and the trailing head may initiate a step. Taken together, these unprecedented behaviors indicate that cytoplasmic dynein achieves processive movement via a mechanism unique among the molecular motors.

2881-Plat
Two Motor Domains of Cytoplasmic Dynein Directly Interact Each Other
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Cytoplasmic dynein is a two-headed molecular motor, which can take hundreds steps processively along a microtubule (MT) without dissociation. This processive movement requires coordination of the two heads, because at least one head must remain bound to a MT. In other two-headed processive motor proteins, it has been reported that intramolecular tension through mechanical elements connecting two motor domains (eg., kinesin's neck linker) plays an