Ca^{2+} sparklets are local elevations in intracellular Ca^{2+} produced by the opening of a single or a small cluster of sarcolemmal L-type Cav1.2 Ca^{2+} channels. At present, however, the spatial organization and mechanisms of modulation of Ca^{2+} sparklets in cardiac myocytes is unknown. Here, we tested the hypothesis that Ca^{2+} sparklets activity varies within the sarcolemma of neonatal cardiac myocytes and that chemically-induced translocation of PKCα increases the Ca^{2+} sparklet activity in these cells. Consistent with this hypothesis we found that application of the PKC activator phorbol 12,13-dibutyrate (PDBu; 500 nM) increased Ca^{2+} sparklet activity in neonatal cardiac myocytes. Analysis of the spatial distribution Ca^{2+} sparklet activity was not random (i.e. did not have a Poisson distribution). Rather, as in reported in smooth muscle cells, Ca^{2+} sparklet activity was higher at specific regions of the cell. Translocation of PKCα to the sarcolemma of neonatal cardiac myocytes resulted in an increase in Ca^{2+} sparklet activity in specific regions of the cell. Data will be presented on the relationship between Ca^{2+} sparklet activity and Ca^{2+} release from the sarcoplasmic reticulum via ryanodine receptors (i.e. Ca^{2+} sparks) during excitation-contraction coupling. Our data suggest that Ca^{2+} sparklet (i.e. L-type Ca^{2+} channels) activity varies within the sarcolemma of neonatal cardiac myocytes and that they are modulated by PKCα, potentially regulating SR Ca^{2+} release during EC coupling in heart.

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Reconstitution of PKA-Dependent Modulation of Cardiac $Ca_v1.2$ Channels

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L-type calcium currents through $Ca_v1.2$ channels initiate contraction in cardiac muscle. Their regulation by neurotransmitters and hormones through second messenger signaling cascades and protein kinase A (PKA) phosphorylation is a key controller of calcium signaling and contractile force. The α_1 subunit C-terminus contains binding sites for multiple regulatory proteins including the PKA/A kinase anchoring protein 15 (AKAP15) complex. The C-terminal domain is proteolytically cleaved but reassociates non-covalently with the truncated channel and acts as a potent autoinhibitor of channel activity. Relief of autoinhibition by cellular regulatory signals acting on the C-terminus provides an attractive mechanism for producing the large increases in calcium current that are observed physiologically. In fact, consistent reconstitution of PKA-dependent regulation of $Ca_v1.2$ channels in non-muscle has been difficult to achieve. To reconstitute such PKA regulation, we optimized the expression of truncated $Ca_v1.2$ channels, the distal C-terminal domain, the $\alpha_2\delta$ subunit, and the β_{2b} subunit to give a functional autoregulatory complex as assessed by whole-cell voltage clamp recordings of tsA-201 cells. Expression of the truncated $Ca_v1.2$ channel with the free distal domain resulted in large decreases in inward barium current and in coupling between voltage-dependent gating and pore opening. We hypothesized that AKAP15 expression would promote PKA association with the distal C-terminal of the channel and increase the likelihood of PKA-dependent phosphorylation. After optimizing AKAP15 expression, currents recorded in 5 μM forskolin were approximately 5-fold larger than those recorded in the presence of kinase inhibitor RO 31-8220 (1 uM). These findings show that the full-range of PKA-dependent modulation of $Ca_v1.2$ channels can be reproduced when an autoinhibitory complex is formed in this manner and provide a substrate for further studies of this physiologically important regulatory process.

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Biochemical and Functional Characterization of Crystallographic Ca2+/CaM-Cav1.2 A-C-IQ Complex Dimer

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The ubiquitous calcium sensor calmodulin (CaM) mediates two important voltage-gated calcium channel (CaV) calcium-dependent modulatory mechanisms through its interaction with the CaV C-terminal tail. Here, we report the structure of Ca2+/CaM bound to a portion of the CaV1.2 C-terminus with three consecutive CaM binding motifs (A-C-IQ domain). The structure reveals two channel chains dimerized via bridging CaMs and interactions between two long, antiparallel helices. Unlike the crystal structure, the CaM peptide complex is a monomer in solution that corresponds to a single channel chain and two CaMs. Disruption of the crystallographic inter-helix interactions had minimal effect on CDI and CDF of full-length CaV1.2. Moreover, subunit counting experiments using CaV1.2-EGFP fusion proteins clearly indicate that CaV1.2 is a monomer in cell membranes. Thus, contrary to previously proposed models, there appears to be no role for dimerization in channel function.

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Enzyme-Inhibitor-Like Tuning of Calcium Channel Connectivity With Calmodulin

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Ca^{2+} channels and calmodulin (CaM) are prominent hubs of signaling networks, extensively coordinated by feedback control. For example, each channel associates with its own CaM (but see *PNAS***106**:5135), acting as a Ca^{2+} sensor that regulates Ca^{2+} entry through channels. Because channels bind CaM avidly, every channel should possess CaM and exhibit regulation, regardless of biological fluctuations of CaM concentration. This would represent a significant form of concentration independence between Ca^{2+} channels and CaM. Here, we reveal significant exceptions to this autonomy, by combining electrophysiology to characterize channel regulation, with concurrent optical FRET sensor determination of free apoCaM concentration in live cells. This approach translates quantitative CaM biochemistry from the traditional test-tube context, into the realm of functioning holochannels within intact cells. From this perspective, we find that long splice forms of $Ca_V1.3$ and $Ca_V1.4$ channels include a distal-carboxy-tail module that functions like an enzyme inhibitor to retune channel affinity for apoCaM. In this configuration, natural CaM variations alter Ca^{2+} feedback gain, and the strength of competitive retuning is customized across channel subtypes ($Ca_V1.3$ versus $Ca_V1.4$), and species (rat versus human $Ca_V1.3$). Given the ubiquity of these channels, the corresponding connections between ambient CaM levels and Ca^{2+} entry via channels are broadly significant for Ca^{2+} homeostasis—presumed alterations of apoCaM levels in neurodegenerative conditions like Parkinson's and Alzheimer's are predicted to increase Ca^{2+} entry, potentially explaining the Ca^{2+} dysfunction underlying these diseases. Mechanistically, our extensions of enzyme-inhibitor analysis argue well that the competitive retuning in holochannels indeed reflects competition between a single distal-carboxy-tail module and a single CaM molecule, both vying for IQ domain occupancy on channels. Finally, our overall approach may be generally useful for the *in situ* analysis of signaling molecules resistant to *in vitro* reconstitution, such as Ca^{2+} channels.

Platform H: Physical Chemistry of Proteins & Nucleic Acids

88-Plat

Single Molecule Observations of DNA Hybridization Kinetics

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Two 25 base-pair complementary DNA strands are encapsulated within an optically trapped nano-droplet, and we observe the kinetics of their hybridization in dynamic equilibrium via single molecule fluorescence resonance energy transfer (FRET) as a function of temperature and of the solution's NaCl concentration. We have directly observed binding and unbinding events between the two freely diffusing DNA strands, and our measurements resolve multiple conformational states at elevated temperatures and low concentrations of NaCl.

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Control of the Viscoelasticity of the Genome By Topoisomerase Type II and Anti-Cancer Drugs

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The mechanical, viscoelastic properties of the genome are important for our understanding of cell division and, indirectly cancer therapy. Topology controlling enzymes (topoisomerase type II) are thought to play an essential role, but so far quantitative measurements of the effect on the viscoelasticity of DNA are lacking. We report experiments showing how double strand passage facilitated by human topo II controls the disentanglement of DNA. For this purpose, we have measured the elastic storage and viscous loss modulus of a model system consisting of bacteriophage DNA in buffer solution using video tracking of the Brownian motion of colloidal probe particles. We found that the viscoelastic response is critically dependent on the formation of entanglements among the DNA molecules with relaxation times on the order of seconds. For the first time we observed that topo II effectively removes these entanglements and converts the system from an elastic gel to a viscous fluid depending on the dissipation of energy through the hydrolysis of ATP. A second aspect of this study is the effect of a generic topo II inhibitor on the viscoelasticity. Topo II inhibitors constitute an important class of anti-cancer drugs, because they impede