occurs within single cells and between cells in tissue, as illustrated during embryonic heart development where cardiac fibroblasts make collagen that cardiomyocytes contract. With few additional assumptions, the basic module has sufficient physics to control key structural genes in both development and disease.

Platform: Ion Channel Regulatory Mechanisms

1836-Plat
Coupling of Distinct Ion Channel Types in Neurons Mediated by AKAP79/150
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M-type K^+ channels, comprised of KCNQ2-5 (Kv7.2-7.5) subunits, play key roles in the regulation of neuronal excitability in the nervous system. In diverse neurons, L-type Ca^{2+} channels (LTCCs) drive transcriptional regulation via NFAT transcription factors, and in sensory neurons, TRPV1 cation channels excite neurons in response to heat, acidity or chemical ligands, driving nociception. The A-kinase-anchoring protein (AKAP)79/150 has been shown to orchestrate regulation of all three types of channels by PKC, PKA, calcineurin and NFATs. Using stochastic optical reconstruction microscopy (STORM) super-resolution microscopy, we have directly visualized individual signaling complexes containing AKAP79/150, these three ion channels and G protein-coupled receptors in neurons and tissue-culture cells. Using multi-color STORM, we observe AKAP150-mediated clustering of KCNQ, LTCCs and TRPV1 channels at the single-complex level. Thus, AKAP79/150 links different channel types together, raising the possibility of their functional, as well as physical, coupling. In sensory neurons, capsaicin caused PIP_2 hydrolysis by TRPV1 activation. In neurons isolated from AKAP150+/+ mice, brief application of low concentrations of capsaicin (100 nM), which we believe triggers only local PIP_2 depletion, induced ~40% suppression of M-current (*IM*), suggesting close localization of TRPV1 and M-channels, the latter thus suppressed by TRPV1-induced local PIP_2 depletion. However, in AKAP150-/- neurons, *IM* was not affected by this modest activation of TRPV1 channels, implying the critical role of AKAP79/150. Application of the LTCC blocker, nifedipine, but not the N-type Ca^{2+} channel blocker, ω-conotoxin GVIA, significantly suppressed desensitization and tachyphylaxis of TRPV1 currents, suggesting the functional coupling of LTCCs with TRPV1 channels, consistent with their physical coupling at the single-complex level seen with STORM. We thus find AKAP79/150 mediates physical and functional coupling of these three ion channels in sensory neurons, indicating physiological roles in tuning the nociceptive response to painful stimuli.

1837-Plat
Stoichiometry of CRAC Channel Assembly and Gating
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CRAC channels are opened by binding of the ER calcium sensor STIM1 to the C-terminus of the channel subunit Orai1. Previous functional experiments suggested a tetrameric channel stoichiometry, but the crystal structure of Drosophila Orai is a trimer of dimers, with each C-terminus forming a coiled-coil with its neighbor. This raises two fundamental questions: what is the stoichiometry of the CRAC channel, and does STIM1 bind to individual or pairs of C-termini to open it? To address these questions, we constructed hexameric concatemers of Orai1. Orai1 hexamers produced currents with properties that were indistinguishable from native ICRAC, including Ca^{2+} selectivity, Ca^{2+}-dependent inactivation, and modulation by 2-APB. The inhibitory effects of single L273D mutations confirmed that all 6 subunits participated equally in forming the functional channel.
STIM1-Orai1 binding was studied using E-FRET between STIM1-YFP and CFP-Orai1. While the Orai1(L273D) C-terminus alone did not bind STIM1, it enhanced binding when paired with a neighboring WT C-terminus. To compare how monomer vs dimer binding are coupled to channel opening, we constructed hexamers with a single truncated or L273D C-terminus. The truncated hexamer showed significantly less activity than the L273D mutant, arguing against a pure monomeric gating mode.
The relationship between STIM1 occupancy and channel activation was examined using Orai1 hexamers containing 1-3 STIM-binding mutations (producing 1-3 Orai1 heterodimers per channel). For both strong (L273D) and weak (L286S) inhibitory mutations, channel activity was well described by a model that assumes independent and equal energetic contributions from each heterodimer.
In summary, we present the first functional evidence that hexameric Orai1 channels have the same properties as native CRAC channels. Our data suggest that STIM1 binds pairs of Orai C-termini and opens CRAC channels as a trimer of dimers, with each dimer contributing a constant amount of gating energy.

1838-Plat
Structure and Selectivity in Bestrophin Ion Channels
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Human bestrophin 1 (hBest1) is a calcium-activated chloride channel from the retinal pigment epithelium, where mutations are associated with vitelliform macular degeneration, or Best disease. We describe the structure of a bacterial homolog (KpBest) of hBest1 and functional characterizations of both channels. KpBest is a pentamer that forms a five-helix transmembrane pore, closed by three rings of conserved hydrophobic residues, and has a cytoplasmic cavern with a restricted exit. From electrophysiological analysis of structure-inspired mutations in KpBest and hBest1, we find a sensitive control of ion selectivity in the bestrophins, including reversal of anion/cation selectivity, and dramatic activation by mutations at the cytoplasmic exit. A homology model of hBest1 shows the locations of disease-causing mutations and suggests possible roles in regulation.

1839-Plat
HCN Channels: The Molecular Basis for their cAMP-TRIP8b Regulation
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Hyperpolarization-activated cyclic nucleotide-regulated (HCN1-4) channels are involved in the regulation of several higher order neural functions, such as short- and long-term memory processes (1). HCN channels are exquisitely sensitive to endogenous levels of cAMP, since they directly bind cAMP through a specialized domain in their cytoplasmic C-terminus (cyclic nucleotide binding domain, CNBD) (2). In addition to cAMP, HCN channels are further regulated by TRIP8b, their brain-specific auxiliary subunit. TRIP8b antagonizes the effect of cAMP on HCN channel opening, as it interacts with the CNBD of the channel (3). We employed solution NMR methodologies to determine the 3D structure of the human HCN2 CNBD in the cAMP-free form, and mapped on it the TRIP8b interaction site. Thus, we were able to reconstruct, for the first time, the molecular mechanisms underlying the dual regulation of HCN channel activity by cAMP-TRIP8b (4). Furthermore, site-directed mutagenesis followed by biochemical/biophysical analysis allowed us to identify key residues within the CNBD involved in TRIP8b binding. These new structural information will provide deeper insights into the molecular basis of neurological disorders associated with dysfunction of the HCN channel conductance in neurons, potentially leading to the design of drugs able to modulate HCN channel mediated memory processes.
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1840-Plat
Live Cell Biochemistry Implicates Protein Kinase a Modulation of L-Type $Ca_V1.4$ Channels
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The regulation of L-type Ca^{2+} channels by protein kinase A (PKA), though biologically crucial, has long remained mechanistically storied and complex, as studied in native cells at one extreme, and through *in vitro* biochemistry at the other. Here, we adopt a different tactic and focus initially on an intermediate context, using ideas drawn from synthetic biology and live-cell biochemistry. We set out to create a form of PKA modulation in L-type channels, based on our recent findings that: (*a*) calmodulin (CaM) competes for binding at a channel C-terminal 'IQ' domain with an 'ICDI' module in the C-terminal extremity