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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

of L-type $Ca_V1.3/1.4$ channels, and (*b*) dislodging CaM profoundly suppresses peak channel opening by severalfold and eliminates their Ca^{2+}-dependent inactivation (CDI) (Adams *et al* (2014), *Cell* **in press**). We reasoned that implanting a synthetic phosphorylation site in ICDI might weaken IQ interaction in a PKA-sensitive manner, allowing channels to rebind CaM and undergo CDI. Cognizant that full-bore PKA signaling is best conserved within certain native rather than model cells, we performed live-cell FRET interaction assays (IQ versus ICDI) in adult guinea-pig ventricular myocytes renown for strong PKA signaling. To our surprise in control experiments, we discovered that IQ interaction with the wild-type ICDI of L-type $Ca_V1.4$ channels is already sharply attenuated by PKA activation, whereas ICDI modules from other L-type isoforms showed no such modulability. Accordingly, we synthesized chimeric L-type $Ca_V1.3$ channels fused to the $Ca_V1.4$ ICDI module, and endowed such channels with robust forskolin-dependent enhancement of CDI, as observed in HEK293 cells. For wild-type $Ca_V1.4$ channels, we now also resolved analogous forskolin activatable CDI. This effect, discovered through a synthetic live-cell biochemical approach, might underlie the dopaminergic regulation of $Ca_V1.4$ implicated in circadian control within the retina.

1841-Plat

A Comprehensive Search for Calcium Binding Sites Critical for TMEM16A Calcium-Activated Chloride Channel Activity

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TMEM16A forms calcium-activated chloride channels (CaCCs) that regulate physiological processes such as the secretions of airway epithelia and exocrine glands, the contraction of smooth muscles, and the excitability of neurons. Notwithstanding intense interest in the mechanism behind TMEM16A-CaCC calcium-dependent gating, comprehensive surveys to identify and characterize potential calcium sensors of this channel are still lacking. By aligning distantly related calcium-activated ion channels in the TMEM16 family and conducting systematic mutagenesis of all conserved acidic residues thought to be exposed to the cytoplasm, we identify four acidic amino acids as putative calcium-binding residues. Alterations of the charge, polarity, and size of amino acid side chains at these sites alter the ability of different divalent cations to activate the channel. Our results thus demonstrate that direct binding of calcium to TMEM16A triggers channel activation independently of calmodulin, identify novel interaction sites between calcium ions and TMEM16A, and lay the groundwork for future studies examining the mechanism of calcium-dependent TMEM16 channel activation.

1842-Plat

Molecular Mechanism of Zinc Inhibition on Voltage-Gated Proton Channel Hv1

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The voltage-gated proton channels (Hv1) have been shown to be involved in many physiological processes in which they play essential roles, such as pH homeostasis and charge compensation. The most potent inhibitor for Hv1 is zinc. For example: the quiescent sperm cells in the male reproductive system become active once introduced into the female reproductive tract by the removal of zinc inhibition. Zinc blocks the Hv1 current by shifting the voltage dependence of channel activation to a more depolarized range. In this study, we use voltage clamp fluorometry technique to identify the molecular mechanism of zinc inhibition on Hv1. We found that the zinc binding site is localized in each subunit of the dimeric Hv1 and that several polar amino acids on the extracellular part of this channel play different roles in the binding. Based on these results, we propose that there exist two sites for zinc occupancy: one is localized close to S1 and affects the voltage dependence of channel opening; the other one is localized in the proximity of the proton permeation pathway and thereby impairing the ion conduction of the channel. Since zinc is by far the only physiological extracellular blocker for Hv1, the detailed study of molecular mechanism of zinc binding will provide valuable information for future drug development for Hv1.

1843-Plat

Alcohol Inhibition of a Chemically-Activated GIRK2 Channel

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Alcohol is widely used and often abused. Yet, the molecular understanding of its action on brain function is poorly understood. Alcohol directly modulates the activity of several ion channels, including the G protein-gated inwardly-rectifying potassium (GIRK) channel. GIRK channels are directly activated by alcohol, independent of their typical G-protein mediated pathway. Currently, however, the molecular mechanism underlying alcohol activation of GIRK is poorly understood. We recently demonstrated that introduction of a Cysteine into the alcohol pocket of GIRK2(L257C) created a channel that could be chemically activated with alcohol-like cysteine-reacting reagents. Here, we studied the channel gating properties of purified GIRK2-L257C channels in a defined reconstituted system that allowed precise control of the lipids, G proteins and ions. We expressed a truncated cys-less GIRK2-L257C(GIRK2Δ*-L257C) in Pichia pastoris, reconstituted purified protein into liposomes and studied the function of purified GIRK2Δ* L257C using a high throughput potassium flux assay. Reconstitution of GIRK2Δ*-L257C into POPE:POPG:PIP2 containing liposomes exhibited a basal, barium-sensitive flux that was potently enhanced by pre-incubation with MTS-hydroxyethyl(MTS-HE) as well as the $G\beta\gamma$ G-protein subunits. Propanol treatment also enhanced the K^+ flux. Thus, in the absence of any other proteins or cytoplasmic regulators, these experiments demonstrate the direct activation of GIRK2 channels by three distinct ligands, $G\beta\gamma$ G-proteins, alcohol and MTS-HE. Interestingly, MTS-HE-activated GIRK2Δ*-L257C channels were inhibited by propanol in a dose-dependent manner. Inclusion of TCEP (tris(2-carboxyethyl)phosphine), which reduces disulfides, decreased MTS-HE activation but converted the propanol response from inhibition to activation. These experiments reveal that GIRK2 channels can also be inhibited by alcohol, perhaps through a different site, depending on the level of basal channel activation. Elucidating the details underlying alcohol's effects on channel proteins is paramount to developing selective pharmacological tools that could be used in the treatment of alcohol abuse and addiction.

Platform: Bioenergetics and Mitochondrial Signaling

1844-Plat

Investigation of the Role of the Phospholipid Cardiolipin in Activating Respiratory Complex Activity

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Cardiolipin is an anionic phospholipid with a unique dimeric structure containing four fatty acids and two phosphate diesters. Within eukaryotic cells, cardiolipin resides predominantly in the energy-transducing mitochondrial inner membrane, where it mediates the assembly of respiratory chain supercomplexes, establishment of cristae morphology, and maintenance of membrane potential. Following its de novo synthesis in the inner membrane, nascent cardiolipin undergoes remodeling to produce a mature form of the lipid with largely unsaturated acyl chains. Abrogation of this remodeling cycle by dysfunction in the transacylase enzyme tafazzin underpins the heritable mitochondrial disorder Barth syndrome. Using an epistasis panel of yeast knockouts in the cardiolipin remodeling pathway, we have shown that remodeled and unremodeled cardiolpin support measurable features of oxidative phosphorylation to a similar extent, despite having marked differences in their acyl chain compositions. This has led us to analyze how physiochemical features other than acyl chain identity - namely the headgroup structure or the absence of a lipid tail - might explain the importance of cardiolipin remodeling. Using respiratory complex IV (cytochrome c oxidase) reconstituted into soluble nanoscale bilayers (nanodiscs) of defined lipid composition, we demonstrate the requirement of cardiolipin in activating respiratory complex redox activity. Moreover, performing fluorescence-based and electrokinetic measurements with model membrane systems, we have analyzed the interaction between divalent cations and cardiolipin-containing bilayers, as well as the proton dissociation behavior of cardiolipin variants. Using modeling that combines Gouy-Chapman-Stern formalism with Langmuir adsorption isotherms, our data indicate that cardiolipin variants bind divalent cations with similar affinities, but differ greatly with respect to cation-dependent alterations in headgroup packing in the bilayer interface. Moreover, our zeta potential measurements challenge the prevailing model that a bicyclic, resonance-stabilized headgroup structure maintains disparate pKa values of the two phosphate groups.