79-MiniSymp

Games Microbes Play: the Game Theory Behind Cooperative Sucrose Metabolism in Yeast

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The origin of cooperation is a central challenge to our understanding of evolution. Microbial interactions can be manipulated in ways that animal interactions cannot, thus leading to growing interest in microbial models of cooperation and competition. In order for the budding yeast *S. cerevisiae* to grow on sucrose, the disaccharide must first be hydrolyzed by the enzyme invertase. This hydrolysis reaction is performed outside of the cytoplasm in the periplasmic space between the plasma membrane and the cell wall. Here we demonstrate that the vast majority (~99%) of the monosaccharides created by sucrose hydrolysis diffuse away before they can be imported into the cell, thus making invertase production and secretion a cooperative behavior [1]. A mutant cheater strain that does not produce invertase is able to take advantage of and invade a population of wildtype cooperator cells. However, over a wide range of conditions, the wildtype cooperator can also invade a population of cheater cells. Therefore, we observe coexistence between the two strains in well-mixed culture at steady state resulting from the fact that rare strategies outperform common strategies_the defining features of what game theorists call the snowdrift game. A simple model of the cooperative interaction incorporating nonlinear benefits explains the origin of this coexistence. Glucose repression of invertase expression in wildtype cells produces a strategy which is optimal for the snowdrift game_wildtype cells cooperate only when competing against cheater cells. In disagreement with recent theory [2], we find that spatial structure always aids the evolution of cooperation in our experimental snowdrift game.

[1] Gore, J., Youk, H. & van Oudenaarden, A., *Nature* **459**, 253 - 256 (2009).
[2] Hauert, C. & Doebeli, M., *Nature* **428**, 643 - 646 (2004).

Platform G: Voltage-gated Ca Channels

80-Plat

Calcium Channel-Activated CREB-Dependent Excitation-Transcription Coupling: Microdomain Organization and Frequency-Dependent Regulation Revealed By Wavelet Analysis

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Voltage-gated $Ca_v1.2$ calcium channels couple membrane depolarization to cAMP response-element-binding (CREB) protein-dependent transcriptional (CDT) activation. To investigate the spatial and temporal organization of CDT signaling microdomains, we combined FRET microscopy with perforated patch clamp technique. The experimental approach to quantitative assessment of CDT signaling evoked by cAMP- and $Ca_v1.2$-dependent mechanisms was developed in COS1 cells expressing recombinant $Ca_v1.2$. Using continuous 2D wavelet transform and time series analyses, we found that nuclear CDT signaling is differentially organized in spatially and temporally separated microdomains of four distinct types. In rat neonatal cardiomyocytes CDT is mediated by cAMP-initiated CaMKII-sensitive and $Ca_v1.2$-initiated CaMKII-insensitive mechanisms. The latter microdomains show tendency to exhibit periodic behavior correlated with spontaneous contraction of myocytes suggestive of frequency-dependent CDT regulation in the heart.

81-Plat

$Ca_v\beta 2$ Subunit Associates with Caveolin-3 and Regulates Trafficking and $\beta_2$adrenergic Receptor Regulation of the Caveolar L-Type Ca^{2+} Channels

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The auxiliary $Ca_V\beta$ subunits ($Ca_V\beta_1$-$Ca_V\beta_4$) influence the trafficking and functional properties of pore forming α subunits of L-type Ca^{2+} channels. Recently we have demonstrated a subpopulation of $Ca_V1.2$ channels in caveolae microdomains in ventricular myocytes that are specifically regulated by the β_2-adrenergic receptor stimulation. We hypothesize that a specific $Ca_v\beta$ subunit isoform is essential for the localization and regulation of caveolar $Ca_V1.2$ channels. Immunogold labeling and electron microscopy demonstrated that $Ca_v\beta_{2c}$ but not $Ca_v\beta_3$co-localized with Cav-3 in ventricular myocytes. GST-Cav-3 pull-down experiments using various Cav-3 domain fusion proteins confirmed that Cav-3 directly associates with $Ca_V\beta_2$ subunit but not with $Ca_V\beta_1$, $Ca_V\beta_3$, or $Ca_V\beta_4$. Immunoprecipitation experiments from transfected HEK293 cells demonstrated that Cav-3 co-immunoprecipitate with $Ca_v1.2$ subunit when coexpressed with $Ca_v\beta_{2c}$ subunit. However, $Ca_v1.2$ did not co-IP with Cav-3 when $Ca_v1.2$ was coexpressed with either $Ca_V\beta_{1b}$, $Ca_V\beta_3$, $Ca_V\beta_4$ or $Ca_v1.2$ subunit alone in HEK293 cells, suggesting $Ca_v\beta_{2c}$ is required for caveolar targeting of $Ca_v1.2$ channels. The functional role of $Ca_v\beta_{2c}$ subunit on caveolar Ca^{2+} channels was analysed by patch-clamp technique in neonatal mouse cardiomyocytes transfected with either a control siRNA or siRNA specific to $Ca_v\beta_{2c}$. In the control siRNA transfected myocytes both β_1AR (norepinephrine, 10uM, prazosin, 10uM) and β_2AR specific (salbutamol, 1uM, atenelol 1uM) stimulation significantly increased $I_{Ca,L}$ by 100% and 60% respectively. However, when the $Ca_v\beta_{2C}$ subunit expression was knocked down by specific $Ca_v\beta_{2C}$ siRNA transfection into the myocytes, the β_2AR specific stimulation of $I_{Ca,L}$ was abolished, where as β_1AR stimulation of $I_{Ca,L}$ was intact. siRNA mediated knockdown of $Ca_v\beta_{2C}$ subunit was confirmed by immunostaining and confocal microscopy. We conclude that $Ca_v\beta_{2C}$ subunit is specifically responsible for the targeting and functional regulation of the caveolar $Ca_v1.2$ channels in ventricular myocytes.

82-Plat

Lysophospholipids Modulate Voltage-Gated Calcium Channel Currents in Pituitary Cells; Effects of Lipid-Stress

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Voltage-gated calcium channels (VGCC) are osmosensitive. To test the hypothesis that this property of VGCCs stems from their susceptibility to alterations in the mechanical properties of the bilayer, we use native VGCCs in pituitary cells and reversibly perturb the bilayer with lipids that alter bilayer stress, i.e. cone-shaped lysophospholipids (LPLs). LPLs of different head group size and charge were used: lysophosphatidylcholine (LPC), lysophosphatidylinositol (LPI), lysophosphatidylserine (LPS) and lysophosphatidylethanolamine (LPE). Phosphatidylcholine (PC) and LPC (C6:0) were used as controls. We show that partition of both LPC and LPI into the membrane of pituitary cells suppressed L-type calcium channel currents (I_L). This suppression of I_L was slow in onset, reversible upon washout with BSA and associated with a depolarizing shift in activation (~ 8mV). In contrast to these effects of LPC and LPI on I_L, LPS, LPE, PC and LPC (C6:0) exerted minimal or insignificant effects. This difference may be attributed to the prominent conical shape of LPC and LPI compared to the shapes of LPS and LPE (which have smaller headgroups), and to PC (which is cylindrical). The similar effects of LPC and LPI on I_L, despite differences in the structure and charge of their headgroups, suggest a common lipid stress mechanism in their action. It is plausible that after slow incorporation of these cone-shaped lipids into the membrane of pituitary cells, bilayer mechanics and consequently lipid-protein interactions are different, in a way that suppresses calcium channel voltage sensor motion and thus positively shifts voltage dependence of activation.

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83-Plat

Interference Between Two Modulators of N-Type (CaV2.2) Calcium Channel Gating Charge Movement

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The ω-conopeptide Prialt has highlighted N-type calcium channels as an important target for the development of drugs to control neuropathic pain. The ω-conopeptides block N-type channels by plugging the pore, but we recently demonstrated that ω-conotoxin GVIA (ωGVIA) could accelerate N-channel Off-gating charge movement (Q_{Off}) and right-shift the Q-V relationship, which our modeling suggested resulted from open state destabilization. R-roscovitine (Rosc) is a purine-based drug that binds to open N-channels to stabilize the open state and slow Q_{Off}. Our model predicted that ωGVIA would interfere with the Rosc effect on N-channels, which provided strong test of our conclusions. Gating currents were recorded in 0.2 mM La^{3+} and 5 mM Mg^{2+} (LaMg) ± 5 μM ωGVIA from N-channels expressed in HEK 293 cells. As predicted, Rosc-induced effects on Q_{Off} were suppressed and shifted to more depolarized voltages. Rosc was able to slow Q_{Off}, but the magnitude of that effect was significantly suppressed by ωGVIA, even at strongly depolarized voltages. The Q_{Off} time constant ($Q_{Off}\tau$) was measured over a range of voltages, and ωGVIA reduced $Q_{Off}\tau$ at each voltage along with the apparent sensitivity of $Q_{Off}\tau$ to voltage. As each of these effects was predicted by our modeling, our results provide additional support for the conclusion that ωGVIA affects N-channel gating by destabilizing the open state. The development of novel drugs that isolate this gating effect from the pore-blocking effect could become effective neuropathic pain treatments with a reduced side effect profile.

84-Plat

Activation of PKC-Alpha Increases Ca^{2+} Sparklet Activity in Cardiac Myocytes

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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.

85-Plat

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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86-Plat

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.

87-Plat

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.

89-Plat

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