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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.

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$Ca_v\beta 2$ 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.

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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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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.

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

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