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neither the lipid targets for this protein nor the residues responsible for binding are well characterized. Our study attempts to identify the respective roles of (a) phosphoinositides such as phosphatidylinositol (4,5)-bisphosphate (PIP2) and (b) anionic backgroundlipids such as phosphatidylserine (PS) in binding of the granuphilin C2A domain to liposomes. Affinities are measured using protein-to-membrane FRET and titration with the competitive inhibitor inositol (1,2,3,4,5,6)-hexakisphosphate (IP6). Granuphilin C2A binds to liposomes containing phosphatidylcholine, dansyl-phosphatidylethanolamine, and PS and/or PIP2. Decreased FRET is measured as the protein is displaced from lipid upon titration with IP6. Granuphilin C2A has similar affinity for liposomes containing either 24% PS or 2% PIP2, but affinity increases ~100-fold in the presence of both target lipids. Binding site(s) for the two lipids are being probed using site-directed mutagenesis and NMR. Overall, granuphilin demonstrates the capability to bind to background anionic lipids and phosphoinositides independently, but the presence of both lipids greatly increases the binding affinity of this protein. This suggests that granuphilin may serve as a coincidence detector to target vesicles to sites of secretion on the plasma membrane.

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The Membrane Bending Action of the Syt-1 C2AB Studied on Supported Lipid Bilayers

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Calcium-triggered exocytosis is vital to the cellular and physiological homeostasis of higher organisms. The broad rules of exocytosis, including its regulation by SNARE proteins and Ca^{2+}, have now been known for several years. However, there is a gap in our knowledge with respect to the molecular events that regulate fusion pore expansion and concomitant content dispersal. Within this context, the studies focus on the Ca^{2+}-binding Synaptotagmin (Syt) family of proteins. Syt actions during exocytosis are often tied to its ability to bend and deform membranes. But very little real-time evidence exists that Syt bends membranes or that membrane bending is an essential part of its function. In this project, we utilized synthetic membranes and purified Syt proteins to answer a specific question: Does Syt bend membranes in a Ca^{2+}-dependent manner? To answer this question, we used a combination of in vitro approaches, including recombinant Syt C2AB proteins, supported lipid bilayers (SLBs), and a curvature-sensitive optical technique called polarized total internal reflection fluorescence microscopy (pTIRFM). Although the results are preliminary, we find that purified Syt proteins added to supported lipid bilayers (SLBs) do create localized membrane deformations in the presence of Ca^{2+}. Future work will investigate how the bending process is regulated, whether it differs between synaptotagmin isoforms, and ultimately, how it relates to the function of synaptotagmin in living cells undergoing exocytosis.

Calcium Signaling I

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Computational System Analysis of Ca^{2+} Signaling in the Pancreatic Beta-Cells

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The Ca^{2+} ion serves as a ubiquitous second messenger in pancreatic beta-cells. Beta-cells are equipped with sophisticated machinery to precisely regulate Ca^{2+} signal in a localization-specific manner to regulate insulin secretion. Based on our mathematical models of beta-cell electrophysiology, Ca^{2+} oscillations, cAMP signaling, metabolism and mitochondrial functions we present an updated complex computational model for beta-cells to clarify the mechanisms underlying intracellular Ca^{2+} responses to varying stimuli. The system employs a Hodgkin-Huxley-type ionic model for plasma membrane action potentials with equations for key currents and pumps including Na^{+}/Ca^{2+} exchangers, TRP and store-operating Ca^{2+} channels. The model incorporates modern data for Ca^{2+} extrusion/uptake systems in the endoplasmic reticulum. The role of mitochondria in regulation of Ca^{2+} signaling is also evaluated. The values of most of the model parameters were inferred from available experimental data. This model simulates and explains the Ca^{2+} dynamics under a wide range of experimental conditions, including changes in islet electrical activity due to ion channel blockade and action of several specific G-protein receptor agonists. Our analysis of the dynamic data provides evidence for a pivotal role of store-operated Ca^{2+} channels in maintenance of physiological cytoplasmic Ca^{2+} concentration even at low glucose levels while voltage-dependent calcium channels are blocked. The role of endoplasmic reticulum, TRP and store-operated Ca^{2+} channels in cytoplasmic Ca^{2+} oscillations was also investigated. This computational systems approach provides a framework to analyze the regulatory mechanisms related to release of calcium stores in beta-cell insulin secretion.

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Acidic Calcium Stores Contribute to Secretory Activity Following Elevation of Camp in the Salivary Gland

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Calcium signaling in response to autonomic neural input drives fluid and protein secretion within salivary glands. Previous research focused primarily on the canonical IP_3-mediated calcium release from ER stores. However, other important reservoirs for calcium release exist such as lysosomes, endosomes, and secretory granules. Whether and how these acidic stores are activated remains largely undefined in terms of their contributions to global calcium signals, protein exocytosis and saliva production in salivary tissue. To address this question we used lysotracker red, a fluorescent dye that labels acidic organelles. Confocal images demonstrated that mouse parotid acini had abundant stores that were apically located and whose pH gradients were dissipated by treatment with the vacuolar H-ATPase inhibitor bafilomycin A1. In contrast, parotid ductal cells displayed only a punctate and dispersed or perinuclear labeling. The functional effect of bafilomycin treatment was tested using live cell imaging methods. Bafilomycin treatment resulted in a two-thirds diminishment of peak calcium release amplitudes evoked by a threshold level of carbachol stimulation. The diminishment in peak amplitude and in the rate of rise of the evoked calcium signal was only evident in cells where cAMP was first elevated by either forskolin or the beta-adrenergic agonist isoproterenol. There is evidence that acidic store calcium release can be triggered by the dinucleotide second messenger NAADP. Consistent with this, NED19, a NAADP receptor antagonist, diminished by half the peak calcium response. This data indicates that calcium release from acidic stores in mouse parotid acinar cells is cAMP-dependent and may be mediated by NAADP. In addition, bafilomycin and NED19 treatment significantly reduced exocytotic secretory activity, even without cAMP elevation, supporting the idea that acidic stores may contribute to vesicle fusion and protein release.

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Mechanics Regulates ATP-Stimulated Calcium Response in Fibroblast Cells

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Cells constantly sense their chemical and mechanical environment. Here we study the effect of mechanics on the ATP-induced calcium response of fibroblast cells in experiments that mimic various tissue environments. We find that in two-dimensional configurations, closely packed cell colonies cultured on a soft polyacrylamide gel (elastic modulus $E = 690$ Pa) have more cells exhibiting calcium oscillations than colonies on a rigid substrate ($E = 36000$ Pa). Cells on soft substrates also show a higher number of oscillations following a stimulation, with a slower decay of calcium level relative to those on rigid substrates. Inhibition of gap junctions results in a decrease of the oscillation period and reduced correlation of calcium responses, which suggests additional complexity of signaling upon cell-to-cell contact. Moreover, the frequency of calcium oscillations is independent of the rigidity of the substrate but depends on ATP concentration and cell-cell contact. In additional experiments with cells embedded in a three-dimensional hydrogel matrix, where cell-to-cell contact is absent, more calcium responses are observed when cells are encapsulated in a softer hydrogel ($E = 85$ Pa). When cell contractility is obstructed by inhibition of myosin, we observe less responsive and fewer oscillating cells. Overall, our observations highlight the influence of mechanical environment on chemical sensing in fibroblast cell colonies.

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Calcium Movement in Cardiac Mitochondria

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