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ERK-Mediated Decoding of Calcium Oscillations in Pancreatic Beta Cells

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Calcium oscillations are thought to underlie pulsatility of insulin secretion from pancreatic beta-cells, which is necessary for maintaining glucose homeostasis. However, it is still unclear how this calcium signal is 'decoded' by other signaling molecules such as protein kinases to modulate functional responses in a context-dependent manner. In this study, we investigated crosstalk between calcium and ERK signaling pathways using FRET-based ERK biosensor, EKAR, and RFP-based calcium biosensor, RCaMP, in live MIN6 pancreatic beta-cells.

We observed that different physiological stimuli elicit different dynamics of ERK activity potentially affecting cell function. Amino acid stimulation resulted in simultaneous transient increase in calcium and ERK activity. Membrane-depolarizing agents, however, resulted in calcium oscillations leading to sustained elevation of ERK activity. Further, we observed that artificial imposition of a train of calcium pulses mimicking ''low-frequency oscillations'' lead to simultaneous low-frequency ERK activity pulses. These results, together with model simulations, suggest that endogenous frequency of calcium oscillations in pancreatic beta-cells may be optimized for sustained ERK activation, potentially leading to efficient insulin gene transcription.

Calcium oscillations were also found to lead to sustained activation of Ras, an upstream signaling molecule in the ERK cascade, indicating that ''processing'' of oscillatory calcium signal was likely mediated by calcium-sensitive RasGEFs and RasGAPs. We show here that calcium-sensitive RasGRF1, RasGRF2 and p120RasGAP are expressed in MIN6 cells which could thus dictate the temporal properties of the signal that is communicated to ERK. Further, we noticed that inhibition of ERK activity resulted in loss of calcium oscillations indicating feedback regulation of input calcium signal by ERK. The presence of this putative feedback loop together with calcium-dependent modulation of ERK activity highlights the intricate cross-regulation of the calcium and ERK signaling pathways in pancreatic beta-cells for precise modulation of functional responses.

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Endoplasmic Reticulum Protein 44 Mediates Cell Migration

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ABSTRACT

Cell motility plays a fundamental role in cancer metastasis. But the intracellular signaling mechanism that regulates cell motility remains unclarified. We have studied the functional role of intracellular Ca^{2+} release channel IP_3 receptors(IP_3Rs) and their novel negatively regulatory protein endoplasmic reticulum protein 44 (ERp44) in A549 cell migration. In wound healing assay, 30 μM 2-APB was used to inhibit Ca^{2+} release through the IP_3Rs, and significantly decreased cell migration rates; while 20 μM Ryanodine did show any effect on cell migration. Immunofluorescence assay showed that cell polarization, membrane protrusion and formation of stress fiber of 2-APB treated A549 cells were inhibited 2h after wound producing. Consistent with this finding, the time-lapse imaging showed A549 cell became less motile after 2-APB treatment. Over expression of ERp44 in A549 cells inhibited 10 μM eATP-induced calcium activates and cell polarization, consequently inhibited cell migration both in wound healing assay and the time-lapse imaging. However, over expression of ERp44 in rat vascular smooth muscle, in which IP_3Rs were not required for cell migration, did not inhibit cell polarization and cell migration. These findings suggest that IP_3Rs are required for A549 cell migration and over expression of ERp44 inhibits cell polarization and cell migration through negatively regulating IP_3Rs.

Key Words Cell migration; ERp44; IP3Rs

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Disease Related Mutations Adjacent to Predicted Multiple Ca^{2+} Binding Sites of Ca^{2+}-Sensing Receptor Altering Intracellular Ca^{2+} Oscillations via Extracellular Calcium and Amino Acid Signaling

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Calcium sensing receptor (CaSR), along other members of the family C G protein-coupled receptors (GPCRs), play very important roles in responding to changes in the extracellular calcium concentrations and in circulating levels of amino acids and integrating these extracellular signals into alterations in intracellular signaling pathways. We have reported several potential calcium-binding sites located within the CaSR's extracellular domain using our developed computational algorithms. In the present study, we first report the differential effects of several disease-related mutations located at the predicted calcium binding sites on the inhibition and activation of intracellular calcium responses using single cell imaging. Interestingly, mutating to different residues at two locations near the hinge region of the ECD could lead to either significantly lose of function of the receptor or gain of function (switch function mutations). Amino acid binding results in differential rescue effect in altering intracellular calcium responses, especially calcium oscillations. We further analyzed the effect of mutation and amino acid binding on the correlation motion, cooperativity, and synergistic activation use computational methods. These results provide important implications for our understanding of how the CaSR integrates information about these two completely different classes of agonists–an inorganic divalent cation, and another hand, a nutrient– how the receptor senses these agonists in healthy and diseased states.

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Ca^{2+} and Mg^{2+} Induced Conformational Changes in Downstream Regulatory Antagonist Modulator (DREAM) Measured by Extrinsic Hydrophobic Probes

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DREAM (downstream regulatory element antagonist modulator) is a multifunctional calcium sensor protein involved in regulation of gene expression and K-channel activity in neuronal cells. Here we present spectroscopic studies of the Ca^{2+} triggered conformational transition in DREAM using the ANS extrinsic hydrophobic probes 1,8-anilinonapthalene and 2,6-anilinonapthalene. Fluorescence emission spectra of the ANS-DREAM complexes show an increase in ANS emission intensity for Ca^{2+} and $Ca^{2+}Mg^{2+}$ bound DREAM consistent with an overall decrease in surface polarity due to the Ca^{2+} binding. From the titration studies the ANS dissociation constants were determined to be: $K_{d1,8\text{-}ANS} = 145 \pm 15$ μM for the apoform and $K_{d1,8\text{-}ANS} = 45 \pm 2$ μM for Ca^{2+}DREAM. Two binding sites for both ANS isomers were identified in apo/Mg^{2+} and $Ca^{2+}/Mg^{2+}Ca^{2+}$ protein using fluorescence lifetime measurements. We propose that one binding site is partially solvent exposed and exhibits a shorter lifetime, $\tau_{1,8\text{-}ANS} = 5.8$ ns and $\tau_{2,6\text{-}ANS} = 6.8$ ns, whereas the second site is buried within the protein matrix with $\tau_{1,8\text{-}ANS} = 16.1$ ns and $\tau_{2,6\text{-}ANS} = 10.9$ ns. Computational simulation of ligand binding support the two site binding model with one site located between EF-3/4 and the second site near Trp_{169} and the entering helix of EF-3. Using fluorescence anisotropy decay two correlation times were resolved for apoprotein and Ca^{2+} bound DREAM. We assign the longer correlation time to a global motion of the protein ($\Phi_{apo/Mg2+} = 55$ ns and $\Phi_{Ca2+/Mg2+Ca2+} = 26$ ns) and the second time to a local motion of the C-terminal domain ($\Phi_{apo/Mg2+} = 13$ ns and $\Phi_{Ca2+/Mg2+Ca2+} = 6$ ns). The 55 ns correlation time is consistent with apo and Mg^{2+}DREAM being in the tetrameric form whereas Ca^{2+} binding to apo or Mg^{2+} DREAM promotes formation of DREAM dimer.

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Mg^{2+}/Ca^{2+} Induced Changes in Structure, Dynamics and Stability of Dream Protein

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Downstream Regulatory Element Antagonist Modulator (DREAM) is a member of the neuronal calcium sensor family that controls activity of potassium voltage channels and regulates c-fos and prodynorphine gene transcription in a Ca^{2+} dependent manner. Here, we have investigated the impact of Mg^{2+} and Ca^{2+} binding on the structure, stability and dynamics of DREAM and DREAM C-terminal domain (DREAM-C). Mg^{2+} binding to the apoDREAM does not alter the secondary or tertiary structure as based on CD and Trp emission spectra whereas the association of Mg^{2+} to either EF-3 or EF-4 in apoDREAM-C results in an increase in protein secondary structure and alteration of the tertiary structure. Ca^{2+} binding to either apo- or Mg^{2+}DREAM and apo- or Mg^{2+}DREAM-C triggers larger conformational changes as evident from the blue-shift in emission spectra and the decrease of Stern-Volmer constant. Ca^{2+} triggered changes in DREAM conformational dynamics were characterized by time-resolved fluorescence. In apoDREAM, single tryptophan residue exhibits two lifetimes (τ_1=3.38 ns, f_1=73% and τ_2=7.72 ns, f_2=27%). Ca^{2+} binding to apo- and Mg^{2+}DREAM leads to the shortening of the first lifetime and decrease of the fractional contribution ($\tau_1 = 1.75$ ns, $f_1 = 47\%$ and $\tau_2 = 7.$ ns, $f_2 = 53\%$). Furthermore, the impact of Ca^{2+}/Mg^{2+} on DREAM stability was determined in equilibrium folding studies. The binding of Ca^{2+} increase