Board B303

Protein kinase C (PKC) can regulate Ca^{2+} sparks in vascular and airway smooth muscle cells (SMCs), but its specific molecular mechanisms remain elusive. In this study, we aimed to determine whether PKCε may regulate Ca^{2+} sparks by interacting with ryanodine receptors (RyRs) and which subtype of RyRs underscores the effect of PKCε in SMCs. Our data indicate that in airway SMCs, inhibition of PKCε by a specific inhibitory peptide or gene deletion significantly increased the frequency of Ca^{2+} sparks, and decreased the amplitude of Ca^{2+} sparks in the presence of xestospogin-C to eliminate functional inositol 1,4,5-triphosphate receptors. PKCε activation with phorbol-12-myristate-13-acetate (PMA) caused a significant decrease in Ca^{2+} spark frequency and increase in Ca^{2+} spark amplitude in the presence of xestospongin-C. The effect of PMA was completely blocked in PKCε$^{-/-}$ cells. RyR1 gene deletion abolished PKCε inhibition-induced increase in Ca^{2+} spark frequency and decrease in Ca^{2+} spark amplitude. The effect of PKCε activation was also prevented in RyR1$^{-/-}$ cells. Modification of RyR2 activity by FK506-binding protein 12.6 gene deletion did not annihilate the effect of PKCε inhibition and activation on either Ca^{2+} spark frequency or amplitude. PKCε inhibition-elicited increase in Ca^{2+} spark frequency and decrease in Ca^{2+} spark amplitude was not eliminated in RyR3$^{-/-}$ cells. RyR3 gene deletion did not inhibit the effect of PKCε activation on Ca^{2+} sparks, either. In conclusion, PKCε regulates Ca^{2+} sparks by specifically interacting with RyR1 in airway myocytes. This novel mechanism to regulate Ca^{2+} sparks may have a physiological importance in SMCs.

Calcium Signaling Proteins

472-Pos Developing Calcium and Proteinase Sensors for Real-time Imaging

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Board B304

We demonstrate the successful design of metal-binding site in several non-metal-binding proteins with desired metal selectivity. More interestingly, these designed proteins retain their native ability to associate with natural target molecules. The solution structure reveals that designed metal binding proteins bind metal ions at the intended site with the designed arrangement, which validates our general strategy for designing de novo metal-binding proteins with multiple functionalities. The structural information also provides a close view of structural determinants that are necessary for a functional protein to accommodate the metal-binding site. Using our design approach, we have developed several fluorescent protein-based sensors with a wide range of affinities that can be applied to monitor calcium signaling at different cellular environments and disease pathways. Different from other available sensors, our developed calcium probes have unique advantages as they do not alter the natural calcium signaling network. In addition, sensors for several different classes of proteinases, such as caspases, thrombin, trypsin, chymotrypsin, have also been developed for real-time imaging. These developed ratiometric sensors are comprised of a single fluorescent protein in contrast to other FRET based sensors which utilized paired fluorescent proteins. They are specifically ideal for monitoring cellular responses at different compartments and quantitative analysis.

Calcium Fluxes, Sparks and Waves

473-Pos Photo-Control of Calmodulin Binding to Target Peptide using Photochromic Compound

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Board B305

Calmodulin (CaM) is a physiologically important Ca^{2+}-binding protein that participates in numerous cellular regulatory processes. CaM has a dumbbell-like shape in which two globular domains are connected by a short α-helix. Each of the globular domains has two Ca^{2+}-binding site called as EF-hand. CaM undergoes a conformational change upon binding to calcium, which enables it to bind to specific proteins for a specific response. In this study, we have demonstrated that photo-control of CaM binding to target peptide using photochromic compound N- (4-phenylazophenyl) maleimide (PAM) which undergoes *cis-trans* isomerization by ultraviolet (UV) - visible (VIS) light irradiation reversibly. PAM was incorporated into CaM mutants that have a single reactive cysteine residue. And we prepared fluorescent fusion protein M13-YFP in order to monitor interaction between CaM and M13 peptide with HPLC using size exclusion column. The binding of PAM-CaM (N60C), PAM-CaM (D64C) and PAM-CaM (M124C) to M13-YFP were apparently photo-controlled by UV-Visible light irradiation reversibly at the appropriate Ca^{2+} concentration. Interestingly, on UV light irradiation, the binding of PAM-CaM (N60C) and PAM-CaM (D64C) increased. Contrary, the binding of PAM-CaM (M124C) was decreased. And on VIS light irradiation, the binding of the PAM-CaM mutants showed opposite effect to UV light irradiation. Currently, we are trying to regulate CaM dependent enzymes using the PAM-CaM reversibly by UV-VIS light irradiation.