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Protein kinase C (PKC) can regulate Ca2+ sparks in vascular and airway smooth muscle cells (SMCs), but its specific molecular mechanisms remain elusive. In this study, we aimed to determine whether PKC ϵ may regulate Ca^{2+} sparks by interacting with ryanodine receptors (RyRs) and which subtype of RyRs underscores the effect of PKCs in SMCs. Our data indicate that in airway SMCs, inhibition of PKCE by a specific inhibitory peptide or gene deletion significantly increased the frequency of Ca²⁺ sparks, and decreased the amplitude of Ca²⁺ sparks in the presence of xestospogin-C to eliminate functional inositol 1,4,5-triphosphate receptors. PKCE activation with phorbol-12-myristate-13-acetate (PMA) caused a significant decrease in Ca²⁺ spark frequency and increase in Ca²⁻ spark amplitude in the presence of xestospongin-C. The effect of PMA was completely blocked in PKCε^{-/-} cells. RyR1 gene deletion abolished PKCs inhibition-induced increase in Ca²⁺ spark frequency and decrease in Ca2+ 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 PKCs inhibition and activation on either Ca²⁺ spark frequency or amplitude. PKCs inhibition-elicited increase in Ca²⁺ spark frequency and decrease in Ca²⁺ spark amplitude was not eliminated in RyR3^{-/-} cells. RyR3 gene deletion did not inhibit the effect of PKCε activation on Ca²⁺ sparks, either. In conclusion, PKCε regulates Ca²⁺ sparks by specifically interacting with RyR1 in airway myocytes. This novel mechanism to regulate Ca²⁺ sparks may have a physiological importance in SMCs.

Calcium Signaling Proteins

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

Jenny J. Yang¹, Jin Zou², Ning Chen², Yun Nancy Huang¹, Mike Kirberger¹, Shen Tang¹, Yubin Zhou¹, Adriana Castiblanco¹, Aldberan Hofer³, April Ellis¹

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

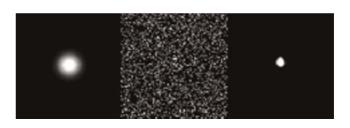
473-Pos Photo-Control of Calmodulin Binding to Target Peptide using Photochromic Compound

Hideki Shishido, Masafumi Yamada, Kazunori Kondo, Shinsaku Maruta

SOKA univ., Tokyo, Japan.

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Calmodulin (CaM) is a physiologically important Ca²⁺-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 Ca²⁺-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 Ca²⁺ 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.



¹ Department of Chemistry, Center for Drug and Advanced Biotechnology, Georgia State University, Atlanta, GA, USA

² Department of Chemistry, Center for Drug and Advanced Biotechnology, Georgia State University, Atlanta, GA, United States, Atlanta, GA, USA
³ Brigham and Women's Hospital, Harvard University, Boston, MA, USA.

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