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Chromophore Formation of Fluorescence Proteins and Its Application of Developing Ca^{2+} Sensors

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Ca^{2+} regulates numerous biological processes through spatio-temporal changes of cytosolic Ca^{2+} concentration and subsequent interactions with Ca^{2+} binding proteins in living cells. There is a strong need to develop Ca^{2+} sensors capable of real-time quantitative Ca^{2+} measurements in specific subcellular environments without using natural Ca^{2+} binding proteins participating in signaling transduction.[1, 2] Taking advantage of fluorescence proteins (FPs) as a useful tool, we created a series of Ca^{2+} sensors by engineering a sensitive location of single FP with different color.[3] Both spectroscopic properties including extinction coefficient, quantum yield, and pK_a and metal binding properties of engineered FPs were identified with different spectroscopic methods including absorbance, fluorescence, and circular dichroism. The engineered Ca^{2+} sensors exhibit a ratiometric fluorescence and absorbance changes upon Ca^{2+} binding with affinities corresponding to the Ca^{2+} concentration found in the ER (K_d values range from 0.4 - 2 mM). The developed Ca^{2+} sensors have applied to monitor Ca^{2+} changes occurring in various subcellular compartments including ER and mitochondria of various mammalian cells upon response of different agonists. These sensors were engineered into virus transfection system for its application to monitor cellular Ca^{2+} signaling during muscle contraction and neuron events.

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Orai1 Channel And Ca^{2+}-independent Phospholipase A_2 Are The New Determinants Of Proliferation And Migration Of Vascular Smooth Muscle Cells

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Ca^{2+} entry is known to play an important role in proliferation and migration of vascular smooth muscle cells (SMC), but the molecular mechanisms that mediate these processes are far from being understood. Here we introduce Ca^{2+}-independent phospholipase $A_2\beta$ ($iPLA_2\beta$) and Orai1-encoded plasma membrane channel (that mediate store-operated Ca^{2+} entry (SOCE) in non-excitable as well as excitable cells) as new molecular determinants of proliferation and migration of aortic SMC. Patch-clamp, Ca^{2+} imaging, immunocytochemistry and molecular approaches were used in this study. We demonstrated that molecular knock down of either $iPLA_2\beta$ or Orai1 (but not TRPC1) channel resulted in full inhibition of store-operated current and Ca^{2+} entry in primary aortic SMC. Transfection of SMC with siRNA to Orai1 impaired their proliferation: Orai1 knock down resulted in $69 \pm 4\%$ decrease in the number of BrDU positive cells and $60 \pm 1\%$ reduction in the rate of their proliferation. Similar effects were observed in $iPLA_2\beta$-deficient cells ($65 \pm 3\%$ and $71 \pm 4\%$ reductions, respectively). Interestingly, transfection of SMC with antisense to TRPC1 did not affect SOCE, but significantly reduced the rate of cell proliferation (by $48 \pm 4\%$). Orai1 and TRPC1 produced additive effects on proliferation, and knock down of both channels resulted in $76 \pm 8\%$ reduction in the rate of SMC proliferation, consistent with different mechanisms for Orai1and TRPC1-mediated Ca^{2+} entry. Molecular knock down of $iPLA_2\beta$, or Orai1 channel also impaired SMC ability to migrate in response to 20% FBS, high glucose, or SERCA inhibition. These results suggest that SOCE mediated by $iPLA_2\beta$-dependent activation of Orai1 channel in SMC is required for their proliferation and migration, and may be involved in vascular SMC responses and angiogenesis triggered by different physiological and pathological conditions.

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Complexity Of Relationship Between STIM1, $iPLA_2\beta$ And Orai1 Expression, Puncta Formation And SOCE Activation In Native And Heterologous Systems

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STIM1, Orai1 and $iPLA_2\beta$ have been identified as crucial elements of the store-operated Ca^{2+} entry (SOCE) pathway, but the mechanism of their functional interaction and molecular requirements for SOCE activation remain controversial. Here we used high resolution imaging, patch clamp, molecular and pharmacological approaches to study functional behavior and mutual relationship between STIM1, Orai1 and $iPLA_2\beta$ in native cells and heterologous systems in which fluorescently tagged STIM1 and/or Orai1 were over-expressed. We found that STIM1 accumulated in puncta equally well in the presence or absence of Orai1, and STIM1 accumulation in puncta is not sufficient for Orai1 accumulation in the same areas. The normal I_{CRAC} could be activated in STIM1-deficient cells. We further found that the effects of C-terminus of STIM1 may be profoundly different in native cells and in cells in which Orai1 and/or full length STIM1 were over-expressed. Also, we found that while inhibition of $iPLA_2\beta$ caused dramatic impairment of endogenous SOCE in native cells, its effects on SOCE in heterologous expressing systems may be less prominent, and may require higher concentrations of inhibitors. Our new data provide first indications of the potential differences in SOCE between native cells and heterologous systems, and challenge the idea of a direct conformational coupling between STIM1 and Orai1 as a mechanism of puncta formation and SOCE activation in native cells.

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Membrane Depolarization of Skeletal Muscle Cells Induces IL6 and SOCS3 mRNA Expression Through Calcium Dependent Stat3 Phosphorylation

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Physical activity has been implicated as a major stimulus for Interleuquin 6 (IL6) expression and release from skeletal muscle in humans. However, the pathways involved in these processes are not well established. Previously, we have reported that potassium depolarization induces IL6 mRNA expression in cultured rat myotubes, Ca^{2+} being an important second messenger for this expression, possibly acting through CREB, NFkB and AP-1.
In other cells systems, STAT3 is a key protein in signaling mediated by IL6, and it has been implicated in the IL6 autocrine up-regulation. Some recent reports have shown a possible calcium regulation of STAT3 tyr 705 phosphorylation, and others have implied GSK3β as a regulator of STAT3 activation; in any case, the mechanisms for STAT3 regulation have not been demonstrated. We report calcium-dependent regulation of STAT3, induced by membrane depolarization as a result of electrical stimulation of cultured rat myotubes. Two peaks of STAT3 phosphorylation (5 and 60 minutes) were identified, when an external electrical field (400 1 ms pulses at 45 Hz) was applied. When myotubes were stimulated with 40 mM Caffeine, a similar STAT3 activation was observed, indicating that Ca^{2+} is necessary for STAT3 phosphorylation. On the other side, overexpression of the Ca^{2+} chelating protein parvalbumin, not only completely inhibited the activation of STAT3, but also diminished by 30% the basal levels of STAT3 phosphorylation.
We also show some preliminary results depicting inhibition of STAT3 phosphorylation by lithium, a well known GSK3β inhibitor, suggesting that this pathway could be playing a role in STAT3 activation by membrane depolarization.
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Isoproterenol-Enhanced Diastolic Sarcoplasmic Reticulum Ca Leak in Ventricular Myocytes Requires Activation of Nitric Oxide Synthase

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We have previously shown increased cardiac ryanodine receptor (RyR)-dependent diastolic SR Ca leak to be present in heart failure (HF) and in conditions when beta-adrenergic (β-AR) tone is high. This SR Ca leak could contribute to the cause of the observed decreased contractility in HF by limiting SR Ca load. Simultaneously, it could also lead to arrhythmogenic Ca-dependent inward depolarizing current commonly seen in failing hearts. We recently demonstrated that this leak increases in manner dependent on calcium-calmodulin-dependent protein kinase II (CaMKII) and completely independent of either protein kinase A (PKA) activation or an increase in bulk free Ca concentration ([Ca]i). Here we investigate this PKA- and [Ca]i-independent activation of CaMKII. We have found that while stimulating intact myocytes with the β-AR agonist isoproterenol (ISO, 250 nM) the CaMKII-dependent enhancement of SR Ca leak is abolished by treatment of the myocytes with nitric oxide synthase (NOS) inhibitor Nω-Nitro-L-arginine methyl ester (L-NAME, 100 μM). When SR Ca load was matched in each group (156 μM), myocytes treated with ISO alone had significantly higher leak (14.2 ± 2.0 μM) vs. those treated with ISO and L-NAME (3.8 ± 1.4 μM) or those left completely untreated