building stable transfected cells and ultimately maximize the efficiency of downstream functional assays.

2766-Pos
Caterpillar Oral Secretion Elicits Reactive Oxygen Species in Isolated Plant Protoplasts

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Over millions of years, plants have evolved and developed several defense strategies such as spines, trichomes, toxins, and plant volatiles to either directly or indirectly defend against insect herbivores. Reactive Oxygen Species (ROS) is a signaling molecule that is suspected of signaling herbivory by activating defense cascade at the cellular level, leading to local and systemic defense gene expression. In this study, we show that tobacco hornworm caterpillar (*Manduca sexta*) oral secretion (OS) induces ROS in tomato (*Solanum lycopersicum*) protoplasts. We used a dye-based ROS imaging approach to dissect the interaction between a damaging herbivore and its host plant. Our study shows that application of Plant-Fed (PF) caterpillar OS increased ROS generation while artificial Diet-Fed (DF) caterpillar OS failed to induce ROS in isolated tomato protoplasts. The elevation in ROS generation was observed after ¯100 s and was saturated after 165 s of PF-OS application. Interestingly, the PF-OS induced ROS increase was abolished in the presence of a Ca^{2+} chelator BAPTA-AM, suggesting possible crosstalk between Ca^{2+} and ROS signaling. Targeting the pathway that triggers ROS generation could be used to develop a herbivore origin potent elicitor of plant defense gene expression (ROS elicitor) pest management research.

2767-Pos
Calcium Buffering by Fluorescent Indicators - Implications and Easy Solutions

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Fluorescent indicators are powerful tools to determine intracellular free Ca^{2+} concentrations ($[Ca^{2+}]$). Although their properties vary widely, indicators share common design and operation principles. Being composed of a Ca^{2+} binding moiety, usually BAPTA-derived, and a fluorophore such as fluorescein, an indicator must bind calcium ions to change its fluorescence. Therefore, within cells, indicators compete with the endogenous buffers for calcium and act as additional buffers, which, in turn, can thwart comparisons of indicator responses and complicate estimating free $[Ca^{2+}]$ within the cell ($[Ca^{2+}]_{in}$).
As lower indicator concentrations tend to report higher $[Ca^{2+}]$, we hypothesized that the true (i.e. indicator-free) $[Ca^{2+}]_{in}$ could be determined by extrapolating the reported $[Ca^{2+}]_{in}$ to the indicator free environment. Although conceptually similar to predecessors, we present a steady state method that avoids the complexities of earlier approaches, and requires only data collected in AM ester-loaded cells. As such, it can be applied to determine the indicator-independent peak $[Ca^{2+}]_{in}$ that is induced by any stimulus in any cell type. To test this approach, we used fura-2 in well-defined *in vitro* systems and found that it estimated not only $[Ca^{2+}]_{in}$, but also, albeit less accurately, the concentration and affinity of endogenous buffers. When applied to data collected in fura-2 loaded neurons depolarized with 50 mM K^+, the method estimated an indicator independent $[Ca^{2+}]_{in}$ in resting (22 nM) and stimulated (1.18 μM) neurons and characterized the endogenous buffer ($\sim$600 μM) as having relatively low affinity ($0.78 \leq Kb \leq 1.63$ μM), results close to those reported previously. While the method does not account for indicator-distorted Ca^{2+} decay rates (a consequence of omitting the time component), it does create a simple and general way to fix peak $[Ca^{2+}]$ amplitudes, the values that are most often measured and compared in routine Ca^{2+} imaging experiments.

Posters: Intracellular Calcium Channels and Calcium Sparks and Waves II

2768-Pos
Defective Interaction of Cam with RyR2 Cam-Binding Pocket Might Contribute to Arrhythmogenic Cardiac Disease

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Ryanodine receptor 2 (RyR2) is a large transmembrane calcium (Ca^{2+}) release channel that mediates Ca^2 release from the sarcoplasmic reticulum to activate cardiac muscle contraction. Calmodulin (CaM) regulation of RyR2 is essential for normal cardiac function. A number of linear fragments of RyR2 have been reported as potential CaM-binding sequences. The sequence 3583-3603aa of human RyR2, which is highly conserved among mammalian isoforms, has been identified as a CaM-binding site in almost all relevant studies and therefore this region is considered as a well-established CaM-binding domain of RyRs. Besides 3583-3603aa region, other RyR2 regions have been also reported as potential CaM-binding sequences. Herein, we used recombinant wild-type CaMprotein and isothermal titration calorimetry (ITC) experiments to screen a number of RyR2-specific synthetic peptides corresponding to the region 4240-4277aa of RyR2, which has been previously proposed as a putative CaM-binding RyR2 region. From all the synthetic peptides screened, a peptide corresponding to 4255-4271aa region of human RyR2 was found to interact with significant affinity with RyR2, in the presence and absence of Ca^{2+} (K_d values 0.60 and 16.58 μM, respectively). Moreover, investigation of the interaction of four arrhythmogenic CaM mutants (N98I, D132E, D134H and Q136P) with this synthetic peptide, as well as the peptide corresponding to the well-established CaM-binding domain of RyR2 (3583-3603aa), revealed that all mutants show disparate binding properties to these two RyR2 peptides, which have been previously proposed to contribute to a putative intra-subunit CaM-binding pocket. Our findings extend our previous observations suggesting that CaM mutations may trigger arrhythmogenic cardiac disease by altering both intrinsic Ca^{2+}-binding, as well as by dysregulating RyR2-mediated Ca^{2+} release via defective interaction of CaM with a distinct CaM-binding pocket that multiple RyR2 regions might contribute.

2769-Pos
RyR2 Hyperactivity Generates Ventricular Tachycardia Susceptibility in Structural Heart Disease

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Ventricular tachycardia/fibrillation (VT/VF) are often responsible for sudden cardiac death in patients with structural heart disease. Antiarrhythmic drug therapy targeting ion channels on the cell surface has limited efficacy and high toxicity. The cardiac ryanodine receptor (RyR2) releases calcium (Ca) from sarcoplasmic reticulum (SR) and is essential for cardiac excitation-contraction coupling. RyR2 hyperactivity due to genetic mutation and/or post-translational modification causes diastolic SR Ca release, resulting in delayed afterdepolarizations (DADs) and triggered beats that can generate ventricular tachyarrhythmia. In mouse models of catecholaminergic polymorphic ventricular tachycardia (CPVT), the mechanistic contribution of RyR2 which mediates catecholamine-induced spontaneous Ca release from SR as a fatal arrhythmia risk is well established. Therefore, we tested the hypothesis that RyR2 hyperactivity plays a key role for VT in structural heart disease and that can be suppressed by RyR inhibitors (Dantrolene and ent-verticilide). To investigate the contribution of RyR2 hyperactivity for susceptibility of inducible VT in structural heart disease, we performed a programmed stimulation using transesophageal ventricular LV pacing protocol in coronary ligation-induced chronic myocardial infarction (MI) mice models of WT and calsequestrin (Casq2)-HET. Although only a small fraction of WT-MI mice exhibited inducible VT (>3 ventricular beats) in basal condition, approximately 50% of MI mice had VT after catecholamine challenge with isoproterenol, which increases RyR2 activity and Ca leak. Sham Casq2-HET mice were non-inducible like WT, whereas Casq2-HET-MI mice exhibited significantly more inducible monomorphic VT compared to WT-MI mice, even though cardiac contractile function on echo and survival were not different. Notably, inducible VT and ventricular ectopy were significantly reduced by pretreatment with RyR2 inhibitors. Hence, our study indicates that increased RyR2 activity can contribute mechanistically to VT induction, and RyR2 may be considered a valuable therapeutic target for preventing VT in structural heart disease.

2770-Pos
Recruiting RyRs to Open in a Ca^{2+} Release Unit

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In cardiac myocytes, clusters of type-2 ryanodine receptors (RyRs) release Ca^{2+} from the sarcoplasmic reticulum (SR) via a positive feedback mechanism