Resolving the Brassinosteroids Signal Transduction Mechanisms by Single-Molecule Assays

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Brassinosteroids (BRs) are the sixth class of plant hormones that involve in numerous plant development processes such as leaf expansion, shoot elongation and pollen tube formation. Once the signal transduction is initiated by the membrane receptor kinase BR1 (brassinosteroid insensitive 1), the signal transmits from the cytoplasm to the nucleus and a number of genes will be regulated. The downstream signaling pathway is realized by three proteins: BIN2 (brassinosteroid insensitive 2), BES1 (BR1 ins suppressor) and a kind of 14-3-3s protein. BRs signaling pathway have been extensively studied via genetics, proteomics, genomics and cell biology techniques. However, these bulk methods can’t follow the transduction process in situ or resolve molecular details at a rate matching the true signaling time-scale. Here we use a single molecule assay based on Total-Internally Reflected Fluorescence (TIRF) microscopy to observe the interaction of these three proteins. The result shows that BIN2 can phosphorylate BES1 in the order of seconds, and the dimer of 14-3-3s can only bind with BES1 in its phosphorylated form. In addition, we have, for the first time, found that the interaction between BIN2 and BES1 is oxygen dependent. This result may have implications on BRs signaling pathway’s involvement of stress acclimation in plants.

Role of Calcium Signaling in Endothelial Barrier Function

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Intact endothelia lining the vasculature play a crucial role in tissue homeostasis and organ function. Various blood borne and tissue released mediators influence endothelial barrier function under physiological conditions and become critical under certain pathologies such as inflammation and sepsis, known to be associated with increased vascular leakage. Prominent inflammatory mediators such as Thrombin and Histamine transiently disrupt the endothelial barrier via activation of G-protein coupled receptors (GPCRs), while other GPCR agonists, including Sphingosine-1-phosphate (SIP), enhance endothelial barrier function.

The barrier disruptive activities of Thrombin and Histamine were repeatedly proposed to be associated with these agonists ability to increase intracellular Calcium (Ca^{2+}) entry through store-operated calcium channels (SOCs) and activation of Gq-associated signaling involving phospholipase C (PLC) activation, production of inositol-1,4,5 trisphosphate (IP3), Ca^{2+} entry through store-operated calcium channels (SOCs) and initiation of Ca^{2+}-dependent endothelial contractility through myosin light chain kinase (MLCK) activation. Here, we use Electric Cell-Substrate Impedance Sensing (ECIS) to challenge this view. We determined barrier function upon stimulation with various barrier modulating agonists in primary human dermal microvascular endothelial cells (HDMECs). We noted obvious discrepancies in the effective agonist concentrations able to evoke...