Some assembly required: SOCE and Orai1 channels couple to NFAT transcriptional activity via calmodulin and calcineurin

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Advances in our ability to monitor the temporal and spatial dynamics of intracellular second messengers such as Ca\(^{2+}\) and cyclic nucleotides at millisecond and sub-micron levels of resolution have greatly increased our understanding of cellular signal transduction mechanisms. Thus, it is now well appreciated that second messengers can rise and fall within discrete regions of the intracellular compartment, as opposed to global changes, and on a time scale determined by the local collection of signaling molecules responsible for the synthesis and degradation/re-uptake of the second messenger. Efforts to identify the components of such macromolecular signaling domains have revealed the presence of hormone receptors, modifying enzymes and scaffolding proteins that tend to assemble and organize these complexes. Emerging evidence now suggests that these signal transduction entities need not be pre-existing, static complexes within the cell, but in fact, may dynamically assemble in response to a specific stimulus. Such an arrangement would thus allow key signaling molecules to be trafficked where they are needed, thereby allowing a cell to utilize these resources more effectively. On the flip side, having such molecules constantly remain within a single cellular domain would facilitate rapid signaling responses and help maintain fidelity of the pathway.

In the current study, Parekh and coworkers investigate how a local elevation in Ca\(^{2+}\) generated by the opening of one or more Ca\(^{2+}\) entry (i.e. CRAC) channels is sensed and utilized by the cell to activate an effector molecule (i.e., NFAT) typically present in the cytosol some distance from the site of Ca\(^{2+}\) elevation. Nuclear factor of activated T-cells (NFAT) is an important transcription factor in immune cells for the \textit{de novo} transcription of cytokines and other mediators of inflammation and is normally maintained inactive in the cytosol as a result of inhibitory phosphorylation. Using single cell fluorescence imaging techniques together with a recombinant protein expression strategy to manipulate key signaling molecules, the authors show that NFAT can be activated by Orai1 channel-mediated Ca\(^{2+}\) entry in response to thapsigargin-stimulated Ca\(^{2+}\) store depletion. The data further implicate the ubiquitous Ca\(^{2+}\) binding protein calmodulin, the Ca\(^{2+}\)-dependent protein phosphatase calcineurin and the scaffolding protein AKAP79/150 in the final signal transduction process. Experimentally, the authors utilized HEK293 cells as a non-excitable cell model that would permit reliable imaging of cytosolic free Ca\(^{2+}\) and NFAT translocation, along with the robust expression of key signaling molecules and NFAT-dependent reporter genes.

Recent work from the Lewis lab\(^1\) has shown that calmodulin can bind to the N-terminus of the Orai1 channel, where it mediates rapid, Ca\(^{2+}\)-dependent inactivation of inward cation current. Point mutations in the N-terminus that disrupt calmodulin binding and Orai1 inactivation were found by Parekh and colleagues to also interfere with the nuclear translocation of NFAT and transcriptional activity following thapsigargin treatment and STIM1-dependent activation of Orai1 channels. These same Orai1 mutations, however, did not alter the nature of thapsigargin-evoked cytosolic Ca\(^{2+}\) transients, and presumably the same is true for Ca\(^{2+}\) elevations generated near the membrane.

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by activated Orai1 channels. Using a reverse immunoprecipitation strategy, the authors found that calmodulin binding to Orai1 (or a closely associated accessory protein) was reduced in the presence of high Ca\(^{2+}\) (e.g., 2 mM), suggesting that Ca\(^{2+}\) entry via open Orai1 channels may promote calmodulin release from the channel. Using TIRF microscopy to image GFP-tagged calmodulin beneath the plasma membrane, the authors further noted that Ca\(^{2+}\) store depletion promoted the formation of calmodulin clusters or puncta that co-localized with Orai1 in the absence of external Ca\(^{2+}\). However, these same puncta dispersed following re-introduction of external Ca\(^{2+}\), suggesting that Ca\(^{2+}\) entry promoted calmodulin dissociation from Orai1 channels.

Whereas Orai1 channels supported store-dependent activation of NFAT, the authors observed that activation of Orai3 channels did not result in NFAT nuclear translocation or transcriptional activity, even though evoked Ca\(^{2+}\) transients were indistinguishable from those generated in cells expressing Orai1 channels. Structurally, the Orai3 channel N-terminus differs from that of Orai1, which was hypothesized to reduce the efficacy of calmodulin binding to Orai3. Biochemical assays revealed that calmodulin binding to Orai3 subunits was considerably weaker compared with Orai1 subunits, thus agreeing with the above speculation. Using mutagenesis to create chimeric channels, the authors replaced the N-terminus of Orai1 with that of Orai3 (N3-Orai1) and mutated Orai3 in a comparable manner (N1-Orai3). Although the N3-Orai1 mutant was not very informative, due to poor expression, the N1-Orai3 chimera did generate normal Ca\(^{2+}\) transients in response to either thapsigargin treatment or the Orai3 selective agonist 2-APB and thus appeared to be functionally intact. Importantly, N1-Orai3 mutant channels supported NFAT activation in cells co-expressing STIM1, suggesting that the N-terminus of Orai1 is sufficient to promote NFAT activity via a related Ca\(^{2+}\) entry pathway. The authors further observed that NFAT nuclear translocation could be driven following activation of a putative heteromeric channel containing Orai1 and Orai3 subunits, suggesting that at least one Orai1 subunit may be sufficient to induce NFAT movement. Biochemically, NFAT activation is dependent upon its dephosphorylation, which is typically catalyzed by the Ca\(^{2+}\)/calmodulin-dependent protein phosphatase calcineurin. Several immunosuppressive agents (e.g., FK506 and cyclosporine) act by inhibiting calcineurin activity, thereby preventing NFAT dephosphorylation, its nuclear translocation and the expression of pro-inflammatory genes. Earlier reports demonstrating that calcineurin can associate with voltage-gated Ca\(^{2+}\) channels prompted the authors to examine the possibility that calcineurin may also interact with Orai1 channels via the scaffolding protein AKAP79. Although no calcineurin appeared to co-localize with Orai1 under basal conditions, an association was detected following Ca\(^{2+}\) store depletion and Ca\(^{2+}\) influx, suggesting that calcineurin may be actively recruited to open Orai1 channels. Knockdown of AKAP79 decreased the association between calcineurin and Orai1 in stimulated cells and also reduced NFAT nuclear translocation. Such observations suggested that AKAP79 transports calcineurin to sites containing clustered STIM1 molecules and activated Orai1 channels, such that stimulated Ca\(^{2+}\) entry would promote the activation/release of calmodulin and its binding to calcineurin. In support, mutation of the calcineurin binding site on AKAP79 also prevented NFAT activation in response to Ca\(^{2+}\) store depletion, suggesting localization of calcineurin at the site of Ca\(^{2+}\) entry to be a critical factor. If Ca\(^{2+}\) entry were simply activating calmodulin and calcineurin in the cytosol, disruption of AKAP79 would be inconsequential.

In the context of Ca\(^{2+}\)-signaling, these data present an interesting variation on the established paradigm of macromolecular signaling complexes by including an active recruitment step to generate functionality. In this scenario, key components (e.g., scaffolding proteins with attached cargo effectors, such as calcineurin and NFAT) participating in the end response are recruited to the STIM1/Orai1 channel complex following store depletion. The mechanistic requirement for calmodulin tethered at the site of Ca\(^{2+}\) entry, along with store depletion-induced recruitment of the cognate target (i.e., calcineurin) and downstream effector (i.e., NFAT) further reduces the risk of calmodulin mis-targeting following its dissociation from Orai1, thereby increasing the selectivity and fidelity of the signal transduction process. Collectively, these data support a mechanism in which local Ca\(^{2+}\) elevations activate effectors typically located some distance from the Ca\(^{2+}\) event, through a process involving active recruitment and spatial co-localization. In cells exhibiting rhythmic Ca\(^{2+}\) oscillations (e.g., mast cells), such a mechanism may prevent the spurious activation of NFAT transcriptional activity by soluble calmodulin and calcineurin.

Previous studies have described neuronal voltage-gated Ca\(^{2+}\) channel activity as a source for local Ca\(^{2+}\)/calmodulin signaling to initiate nuclear transcriptional activity and the picture emerging from the current study appears to be another variation of this signaling platform. A key question arising from this study is how Ca\(^{2+}\) store depletion triggers the dynamic recruitment of AKAP79/calcineurin to activated STIM1/Orai1 channel complexes. Finally, additional studies in native cells and tissues will help validate the key tenets of this SOCE- and Ca\(^{2+}\)/calmodulin-driven signaling mechanism and establish its physiologic importance in non-excitable cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Reference

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