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Conformational dynamics of STIM1 activation

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The binding of stromal interaction molecule 1 (STIM1) to ORAI calcium channels is critical for store-operated calcium entry (SOCE), a calcium influx pathway conserved in virtually all vertebrate organisms. Although many of the major steps of this process are well-understood, crucial details regarding the mechanism of STIM1 activation are unclear. A new study by Zhou et al. provides important new insights into the conformational dynamics of STIM1 activation.

STIM1, and its homologue STIM2, are Ca^{2+} binding proteins located in the membrane of the ER ^{1,2}. Their N and C termini are located in the ER lumen and cytoplasm, respectively, and these two domains function to sense the luminal ER Ca^{2+} concentration and activate ORAI calcium channels in the plasma membrane (PM) (Figure 1) ^{3,4}. Stimulation of cells via receptors at their surface results in depletion of Ca^{2+} from the ER and the dissociation of Ca^{2+} from an EF-hand in the N terminus of STIM1 (STIM1^{NT}) that is located adjacent to a sterile alpha motif (SAM). In cells with replete ER Ca^{2+} stores, the EF-SAM domain exists in a closed configuration ⁵. Upon Ca^{2+} release from the ER, hydrophobic regions in the EF-SAM domain are exposed, thereby allowing neighboring STIM1^{NT} to dimerize ^{5,6}. In fact, forced dimerization of STIM1^{NT} using a rapalog system is sufficient to initiate STIM1 activation ⁷. Activated STIM1 translocates within the ER to ER-PM junctions where it accumulates into discrete puncta. Here, STIM1 binds to ORAI1, the pore-forming subunit of the Ca^{2+} release-activated Ca^{2+} (CRAC) channel ⁸, trapping ORAI1 channels into puncta, and enabling localized Ca^{2+} influx ⁹. STIM1 puncta formation and ORAI1 activation critically depend on STIM1 oligomerization, a process mediated by coiled-coil (CC) domains in STIM1^{CT} ^{10,11}. Three such domains, CC1–CC3, are present in STIM1^{CT} and two of them, CC2 and CC3, are part of a ~ 110 amino acid domain, which is required for STIM1 oligomerization following store depletion. This domain is alternatively called CRAC activation domain (CAD) ¹², STIM1-ORAI activating region (SOAR) ¹³ and Coiled-coil domain b9 (CCb9) ¹⁴, and its expression as a soluble protein is sufficient to activate CRAC channels ^{12–15}. The recent crystal structure of the human SOAR domain reveals a V-shaped homodimer with the CC2 and CC3 domains forming a hair-pin motif. (Figure 1) ¹⁶. Collectively, these data suggest a model of STIM1 activation wherein in cells with replete Ca^{2+} stores, full-length STIM1 is partially dimerized via interactions of its cytoplasmic SOAR domain whereas its ER luminal N terminus exists in a folded and Ca^{2+} -bound monomeric configuration ⁵.

Two overlapping questions regarding the mechanisms underlying STIM-mediated activation of ORAI channels arise from these findings. (1) How does dimerization of STIM1^{NT} result in conformational changes in STIM1^{CT} that enable activation of ORAI channels? (2) Why is STIM1 inactive in cells with replete Ca²⁺ stores although its SOAR domain appears to be already dimerized and has the potential, at least as a soluble protein, to activate ORAI channels? Mechanisms must be in place that restrain SOAR function in the context of full-length STIM1 in cells with replete Ca²⁺ stores?

Zhou et al. now use elegant biophysical approaches to elucidate the conformation alterations in STIM1^{CT} to examine these questions. They show that dimerization of the membrane proximal CC1 domain of STIM1^{CT} stabilizes the α -helical structure of CC1 and undocks SOAR from a binding site on CC1. This results in the exposure of a polybasic domain and an extended conformation of STIM1^{CT} that likely allows it to bridge the gap between the ER and PM and to interact with ORAI channels and phospholipids in the PM (Figure 1).

In order to activate ORAI channels in the PM, STIM1^{CT} has to bridge a distance of 10–25 nm, the estimated width of ER-PM junctions^{17,18}. Previous studies have suggested that a conformational change resulting in the unfolding of STIM1^{CT} is required to achieve this feat^{19,20}. Using lanthanide-based resonance energy transfer (LRET), Zhou et al. analyzed the conformation of STIM1^{CT} under conditions that mimic resting or activated forms of STIM1. They placed the Tb³⁺ LRET donor and a fluorescent acceptor at opposing ends of STIM1^{CT} and analyzed energy transfer in wildtype STIM1^{CT} and a L251S mutant that was previously shown to activate STIM1 function²⁰. Significant LRET is seen only in wildtype but not L251S mutant, which supports the conclusion that wildtype STIM1^{CT} assumes a folded, inactive conformation in which the N and C terminal ends of STIM1^{CT} are located close to each other (Figure 1). Mutation of L251 to serine abolishes this interaction, resulting in an elongated conformation of STIM1 that may be able to bridge the ER-PM junction gap. This interpretation is consistent with FRET studies using a shorter cytoplasmic STIM1 fragment (aa 233–474) which identified the STIM1 activating L251S mutation²⁰.

An important, unresolved question is how dimerization of the ER luminal STIM1^{NT} after depletion of intracellular Ca²⁺ stores affects STIM1^{CT} and enables activation of ORAI channels. This is an important issue because the cytoplasmic SOAR domain of STIM1 appears to be already dimerized¹⁶ and therefore theoretically ready to bind to ORAI^{12–14}. What mechanisms prevent this from happening in full-length STIM1 in non-stimulated cells? Zhou et al. use LRET measurements to demonstrate that the CC1 domains in soluble STIM1^{CT} proteins are not closely associated since LRET was observed only when CC1 domains were forceably crosslinked. Interestingly, the study finds that soluble monomeric CC1 does not form a stable α -helix, whereas crosslinking of CC1 domains significantly increased their α -helical content, thereby further facilitating their dimerization. At first glance, the conclusion that the CC1 domains are not closely associated contrasts with an earlier finding that CC1 supports the resting self-association of full-length STIM1¹¹. However, in the model proposed by Zhou et al. apposition of CC1 domains in the context of full-length STIM1 is facilitated by the dimerization of the luminal EF-SAM domains following store depletion. Therefore, much like a zipper, the dimerization of STIM1^{NT} would be predicted to alter the structure of cytoplasmic CC1 domains and trigger their

dimerization. An important consequence of these changes is that the elongated α -helical CC1 dimer is likely to push the SOAR dimer towards the PM, bringing it into close proximity of ORAI channels.

What other effects does CC1 self-association have on the conformation and activation status of STIM1^{CT}? Previous studies have shown that CC1 contains inhibitory domains that restrain STIM1 activation by interacting with residues in SOAR. For instance, an acidic domain (aa 302-322) in CC1 was reported to interact with a polybasic domain (aa 382-386) in SOAR, thereby keeping STIM1 in an inactive state and preventing ORAI1 activation¹⁹. An inhibitory α helix (aa 310-337) in CC1 was identified by Yang et al. and shown to interact with residues at the base of the SOAR dimer¹⁶. Deletion of the inhibitory α helix resulted in constitutive colocalization of STIM1 with ORAI1 and Ca²⁺ influx¹⁶. Zhou et al. provide intriguing new data that support this model of CC1-mediated inhibition of SOAR function. Analyzing the interaction of monomeric and dimeric (i.e. crosslinked) CC1 with the isolated SOAR, they find that monomeric, but not dimeric, CC1 efficiently binds SOAR when immobilized on amylose resin. The conclusion that CC1 dimerization interferes with SOAR binding to CC1 is also strongly supported by findings in their study that disulfide crosslinking of CC1 abolishes LRET between donor/acceptor pairs placed at opposing ends of STIM1^{CT}, arguing that CC1 dimerization changes the conformation of STIM1^{CT} from a closed to open state.

The results of this study provide detailed mechanistic insights into the activation-induced conformational changes in STIM1^{CT} and provide a consistent picture of how apposition of CC1 domains, triggered by depletion of ER Ca²⁺ stores and dimerization of the ER luminal STIM1^{NT}, unfolds STIM1^{CT} and releases inhibitory interactions between CC1 and SOAR that permit SOAR to bind to ORAI channels. Of course, these results also raise several fundamental questions about how CAD is exposed following STIM1^{CT} extension, the nature of the tertiary structures of STIM1^{CT} in the resting and active states, and whether there are alterations in SOAR itself during STIM1 activation. Further structural and function approaches will be necessary to resolve these questions.

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Figure 1. Model of the structural dynamics underlying STIM1 activation and gating of ORAI channels

Stimulation of cells by surface receptors results in the activation of phospholipase C, production of IP₃ and IP₃ receptor-mediated release of Ca²⁺ from ER Ca²⁺ stores. The resulting fall in [Ca²⁺]_{ER} triggers store-operated Ca²⁺ entry (SOCE) via the activation of stromal interaction molecule 1 (STIM1) and opening of ORAI calcium channels. In resting cells with replete stores, Ca²⁺ ions bound to a canonical EF-hand in STIM1^{NT} stabilize the binding of the EF-hand to a neighboring sterile alpha motif (SAM), preventing EF-SAM from engaging in protein-protein interactions (left)⁵. Upon Ca²⁺ store depletion, the EF-SAM domain unfolds, thereby allowing neighboring EF-SAM domains to dimerize (right)^{5,6}. While the crystal structures of Ca²⁺-bound and Ca²⁺-free STIM1^{NT} have been solved, the structure of STIM1^{CT} is only partially known. STIM1^{CT} contains 3 coiled-coil domains, CC1-CC3, two of which (CC2, CC3) are part of a critical STIM1-ORAI activating region (SOAR)^{12–14}. The crystal structure of the human SOAR reveals four α helices (α1–α4) arranged in a hairpin structure, with α1 and α4 corresponding roughly to CC2 and CC3, respectively¹⁶. SOAR forms a homodimer with a V-like structure held together by hydrophobic interactions and hydrogen bonds at the base of the V-shaped dimer. It is likely that in cells with filled Ca²⁺ stores, STIM1 is partially dimerized via interactions of its cytoplasmic SOAR domain, whereas its ER luminal N terminus is a monomer. The study by

Zhou et al. demonstrates that the CC1 domains in STIM1^{CT} have a low α helical content and do not self-associate. Crosslinking of CC1 domains *in vitro* or their expected apposition following dimerization of STIM1^{NT} *in vivo* stabilizes their α helices and facilitates their self-association. In addition, CC1-CC1 association weakens the binding of SOAR to CC1 and results in the unfolding of the STIM1^{CT}. Together, these structural changes result in the elongation of STIM1^{CT}, allowing it to bind to ORAI channels and anionic phospholipids in the plasma membrane and to activate SOCE. IP₃, inositol 1,4,5-triphosphate; PIP₂, phosphatidylinositol 4,5-bisphosphate; K, lysine-rich region.