Dynamic Coupling of the Putative Coiled-coil Domain of ORAI1 with STIM1 Mediates ORAI1 Channel Activation

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STIM1 and ORAI1 (also termed CRACM1) are essential components of the classical calcium release-activated calcium current; however, the mechanism of the transmission of information of STIM1 to the calcium release-activated calcium/ORAI1 channel is as yet unknown. Here we demonstrate by Förster resonance of STIM1 to the calcium release-activated calcium/ORAI1 components of the classical calcium release-activated calcium current as well as functional communication with STIM1 and failed to impede coiled-coil domain formation lacked both interaction and entry. The cytosolic STIM1 C terminus itself was able, in vitro as well as in vivo, to associate with ORAI1 and to stimulate channel function, yet without ORAI1-STIM1 cluster formation. The dynamic interaction occurred via the C terminus of ORAI1 that includes a putative coiled-coil domain structure. An ORAI1 C terminus deletion mutant as well as a mutant (L273S) with the most widespread and perhaps primordial route for Ca2+ entry across the cell membrane is through store-operated channels (SOCs), which serve essential functions from secretion to gene expression and cell growth (1). The prototypic SOC is the Ca2+ release-activated Ca2+ (CRAC) channel. It is activated by depletion of intracellular Ca2+ stores, which is induced by the second messenger inositol 1,4,5-triphosphate (2–7). The signaling events leading to the activation of CRAC/SOC as well as the composition of this channel have been a long standing mystery. Recently the use of function-based genetic screen by systematic RNA interference provided solid evidence for a key role of STIM (stromal interaction molecule) and ORAI (also termed CRACM) as essential components of SOC (8–10).

STIM1 has been identified as a Ca2+ sensor and regulator of the store-operated Ca2+ influx and CRAC function. These proteins are associated in the ER membrane and sense Ca2+ via an EF hand Ca2+-binding site located in the lumen of the ER. Store depletion triggers a rapid redistribution of STIM1 into ER puncta close to the plasma membrane suggested to activate CRAC/SOC channels (11–13).

RNA interference-based screen as well as analysis of single nucleotide polymorphism arrays of patients with severe combined immune deficiency syndrome who are defective in CRAC function have identified the protein ORAI1. Severe combined immune deficiency syndrome patients are homozygous for single missense mutation in ORAI1, i.e. ORAI1R91W, leading to the loss of store-operated Ca2+ entry (10). ORAI1 is a plasma membrane protein with four predicted transmembrane segments containing a putative coiled-coil motif (14, 15), a common protein interaction domain, in the C terminus.

Coexpression of STIM1 and ORAI1 revealed their colocalization at ER-plasma membrane junctions (10, 14, 16–22), which comes along with the activation of Ca2+ currents representing similar biophysical and pharmacological properties following Ca2+ store depletion like endogenous CRAC currents in RBL mast and Jurkat T cells (15). Based on permeability studies of different point mutations in transmembrane regions 1 and 3 of ORAI1, it is suggested to form the pore of store-operated channels (15, 23). Subsequently both proteins are supposed to manifest the main components of CRAC; however, the detailed mechanism between STIM1imerization and ORAI1 activation is still unknown.

The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S3.

5 The abbreviations used are: SOC, store-operated channel; FRET, Förster resonance energy transfer; CRAC, Ca2+-release-activated Ca2+; TG, thapsigargin; ER, endoplasmic reticulum; GST, glutathione S-transferase; CFP, cyan fluorescent protein; YFP, yellow fluorescent protein; HEK, human embryonic kidney.
To elucidate the communication between STIM1 and ORAI1, we utilized Förster resonance energy transfer (FRET) microscopy to reveal a dynamic coupling of STIM1 and ORAI1 within a range of <10 nm, which results in the activation of Ca$^{2+}$ entry. STIM1-STIM1 multimerization is followed by STIM1-ORAI1 interaction with a significant delay in time, and both processes could be reversed by store refilling. This association was also observed with the C terminus of STIM1 alone, but in a constitutive manner that resulted in channel activation yet without ORAI1-STIM1 cluster formation. A putative coiled-coil structure in the C terminus of ORAI1 was identified as the relevant domain for this functional protein-protein coupling, whereas the N terminus together with the critical position of Arg$^{91}$ controlled rather the gating than the interaction with STIM1.

**EXPERIMENTAL PROCEDURES**

**Molecular Cloning and Mutagenesis**—Human ORAI1 (ORAI1; accession number NM_032790) was kindly provided by A. Rao’s lab (Harvard Medical School). C-terminally tagged pECFP-N1 and pEYFP-N1/ORAI1 constructs were cloned using the Xhol and BamH1 sites of the contemplated vectors. N-terminally tagged ORAI1 constructs were cloned via Sall and SmaI restriction sites of pECFP-C1 and pEYFP-C1 expression vectors (Clontech). An N-terminal deletion mutant (ΔN-term ORAI1, amino acids 89–301) was cloned via PCR into the T/A site of pCDNA3.1V5-His TOPO expression vector (Invitrogen) and recloned into pECFP-C1 and pEYFP-C1 internal restriction sites KpnI and XbaI. A C-terminal deletion mutant (ORAI1-ΔCterm, amino acids 1–260) was constructed similarly, and ECFP-C1 as well as EYFP-C1 N-terminally labeled ORAI1-ΔCterm constructs were prepared as described above. pECFP/pEYFP-C1/ORAI1 served as a template for the generation of the coiled-coil mutant L273S and the ORAI1 R91W mutant. Suitable primers exchanged the corresponding codon from GAG to TCG (L273S) or from CGG to TGG (R91W) using a QuikChange XL site-directed mutagenesis kit (Stratagene). Human STIM1 (STIM1, accession number NM_023790) was provided by T. Meyer’s lab (Stanford University). C-terminally (Stratagene). Human STIM1 (STIM1, accession number NM_023790) was provided by T. Meyer’s lab (Stanford University). C-terminally tagged pEYFP-C1/ORAI1 were synthesized in separate reactions using the TNT expression system (Promega) with STIM1 C terminus employed as a prey was tested for its ability to interact with prebound ORAI1 fragments. GST alone served as a negative control to detect possible nonspecific interaction of His-tagged STIM1 C terminus, which did not occur in any experiment (data not shown). GST fusion proteins were expressed in *Escherichia coli* and purified with the GST gene fusion system (GE Healthcare). His-tagged STIM1 C terminus was obtained from transiently transfected HEK293 cells and purified using the MagneHis protein purification system (Promega). Glutathione-Sepharose was incubated with 20 μg of GST fusion proteins for 30 min at 4 °C. To eliminate nonspecific binding, the samples were washed three times according to the manufacturer’s manual. Purified His-tagged STIM1 C terminus (80 μg) was then incubated with the GST fusion proteins at 4 °C for 80 min. After incubation, Sepharose was washed three times, and bound proteins were eluted by the addition of 360 μl of elution buffer. Eluted proteins (40 μl) were subjected to electrophoretic separation on a 12% SDS-PAGE. To visualize potential interaction of His-tagged STIM1 C terminus and GST fusion proteins, Western blot analysis was carried out using a His$_{6}$ horseradish peroxidase-conjugated antibody (Qiagen). Inputs of GST fusion proteins were detected using the blotted membrane with an anti GST antibody (GE Healthcare).

**Electrophysiology**—Electrophysiological experiments were performed at 20–24 °C, using the patch clamp technique in the whole cell recording configuration. For STIM1/ORAI1 current measurements, Pipette solutions were composed of 3.5 mM MgCl$_2$, 145 mM cesium methane sulfonate, 8 mM NaCl, 10 mM HEPES, 10 mM EGTA, pH 7.2. Extracellular solution consisted of 145 mM NaCl, 5 mM CsCl, 1 mM MgCl$_2$, 10 mM HEPES, 10 mM glucose, 10 mM CaCl$_2$, pH 7.4.

**Confocal FRET Fluorescence Microscopy**—Confocal FRET microscopy was performed similarly as described in Ref. 24. In brief, a QIC100 real time confocal system (VisiTech Int.) was used for recording fluorescence images connected to two Photometrics CoolSNAP HQ monochrome cameras (Roper Scientific) and a dual port adapter (dichroic, 505lp; cyan emission filter, 485/30; yellow emission filter, 535/50; Chroma Technology Corp.). This system was attached to an Axiovert 200M microscope (Zeiss) in conjunction with an argon ion multi-wavelength (457, 488, and 514 nm) laser (Spectra Physics). The wavelengths were selected by an Acousto Optical Tuneable Filter (VisiTech Int.). MetaMorph 5.0 software (Universal Imag-
ing Corp.) was used to acquire images and to control the confocal system. Illumination times for CFP/FRET and YFP images that were recorded with a minimum delay consecutively were approximately 900 ms. Prior to the calculation, the images have to be corrected because of cross-talk as well as cross-excitation. For this, the appropriate cross-talk calibration factors were determined for each of the constructs on the day the FRET experiments were performed. The corrected FRET image ($N_{FRET}$) was calculated after background subtraction and threshold determination using a custom-made software (25) integrated in MatLab 7.0.4 according to the published method (26). The local ratio between CFP and YFP might vary because of different localizations of diverse protein constructs, which could lead to the calculation of false FRET values (27). Accordingly, the analysis should be limited to pixels with a CFP:YFP molar ratio between 1:10 and 10:1 (27) to yield reliable results. This occurred rather seldom in our experiments, and approximately 90% of ratios are between 1:5 and 5:1.

Statistics—Significance analysis was performed with the two-tailed Mann-Whitney test. The mean ± S.E. values are shown throughout the manuscript.

RESULTS AND DISCUSSION

Coupling between STIM1 and ORAI1—Association of ORAI1 and STIM1 in a signaling complex is a controversial issue (19, 28, 29). To evaluate the possibility of a dynamic, physical interaction as a concept of communication between STIM1 and ORAI1, we utilized confocal FRET microscopy for imaging of living human embryonic kidney 293 cells that expressed both proteins in fluorescent-labeled form. These proteins either N- or C-terminally tagged with CFP (donor) or YFP (acceptor) displayed similar behavior as native proteins (data not shown), evident from the activation of CRAC-like currents following ER store depletion (Fig. 1a). Their biophysical characteristics such as time course of activation and maximum current density were not significantly different from native CRAC currents, with a reversal potential larger than $+50 \text{ mV}$ (Fig. 1b). ORAI1 has been reported to redistribute in response to store depletion from a homogenous to a clustered localization accumulating in the plasma membrane adjacent to clustered STIM1 (18, 21). Following ER store depletion by thapsigargin, we observed (Fig. 1c, left panel) both proteins labeled C-terminally colocalized in clusters. Such regions exhibited robust FRET values ($0.09 \pm 0.01$, $n = 8$; Fig. 1g) demonstrating high local proximity of the tagged proteins, compatible with either direct or indirect interaction between STIM1 and ORAI1. In control experiments in which STIM1 was labeled at its N-terminal side, which is located within the ER lumen, no FRET was measured ($-0.01 \pm 0.01$, $n = 10$; Fig. 1, c, right panel, and g) consistent with distant localization of fluorescence labels on opposite sides of the membrane. It is of note that in this situation a similar STIM1-ORAI1 cluster formation is evident (Fig. 1c, right panel).

STIM1 C Terminus Interacts in Vivo as Well as in Vitro with ORAI1—For further investigation of the interaction, we utilized the C terminus of STIM1, which is expected (13) to constitutively couple to ORAI1 provided that no other processes emerging from ER store depletion are absolutely required for channel activation. Coexpression of ORAI1 with STIM1 C-terminus resulted indeed in constitutive currents (Fig. 1d) with current-voltage relationships (Fig. 1e) similar to Fig. 1b. Activation of these currents by STIM1 C terminus occurred to a similar extent when endogenous STIM1 was suppressed by small interfering RNA specific to full-length STIM1 (data not shown). N-terminally tagged STIM1 C terminus displayed, besides some cytosolic localization, clear targeting to the plasma membrane when expressed together with ORAI1 in HEK cells (Fig. 1f) but remained cytosolic when expressed alone (Fig. 1f; lower panel). STIM1 C terminus was colocalized with ORAI1 and yielded robust FRET (0.074 ± 0.007, $n = 36$). Generation of clusters was not visible when only the C-terminal fragment instead of full-length STIM1 was used (Fig. 1, compare c and f), underscoring the ER localization of the latter as a prerequisite for cluster formation (in this process). Nevertheless, the STIM1 C terminus is principally sufficient as a surrogate of STIM1 in terms of association and channel activation, yet independent of ER store depletion and ORAI1 cluster formation. In vitro pulldown experiments using His$_{6}$-tagged STIM1 C terminus as bait demonstrated the ability of the C-terminal domain of STIM1 to associate with ORAI1 in cell-free conditions (Fig. 1h, left panel). ORAI1 binding increased significantly (33 ± 4 versus 23 ± 5 arbitrary units, $n = 9$, $p < 0.01$) above the relatively high background binding of ORAI1 when His$_{6}$-tagged STIM1 C terminus was prebound. To strengthen this in vitro approach, we focused on the N and C termini of ORAI1 and employed these fragments as GST proteins to potentially pull down His$_{6}$-tagged STIM1 C terminus (Fig. 1h, right panel). The C terminus of ORAI1 in contrast to its N terminus showed clear interaction with STIM1 C terminus ($n = 7$), indicating association of the proteins in vitro and suggesting binding as a molecular process involved in channel activation.

Dynamic STIM1-ORAI1 Coupling Is Controlled by the Filling State of the ER—To analyze kinetic aspects of this coupling process, we monitored the time dependence of the STIM1-ORAI1 interaction by dynamic FRET measurements in response to ER store depletion by thapsigargin or ionomycin (Fig. 2a). Under resting conditions the STIM1-ORAI1 interaction seemed to be largely reduced (Fig. 2a) based on the low FRET values. Despite some colocalization between STIM1 and ORAI1, clusters were scarcely visible. Following thapsigargin application, formation of STIM1-ORAI1 coclusters developed over a time period of 240 s and was temporally as well as spatially correlated with an increase in FRET (Fig. 2, a and b). This suggested a causal relationship between ER depletion and development of dynamical STIM1-ORAI1 interactions. Consistently, ionomycin (Fig. 2b, see also Fig. 2g), which is known to deplete ER stores more rapidly than thapsigargin, induced both a faster onset and development of FRET over 120 s, further substantiating that the increasing STIM1-ORAI1 interactions are linked to store depletion.

To further elucidate this relationship, we imaged individual steps of the STIM1-ORAI1 activation machinery over time at several sequential stages: Ca$^{2+}$ depletion of ER, STIM1 multimerization, STIM1-ORAI1 interaction, and homomeric ORAI1 complex formation. The time course of ER store depletion was monitored employing ER-targeted cameleon (31) (YC4-ER)
FIGURE 1. Full-length STIM1 as well as its C terminus interact with ORAI1 leading to CRAC-like currents. a, time course of whole cell inward currents at −74 mV activated by passive store depletion of HEK293 cells expressing labeled STIM1 and ORAI1 as indicated ($t = 2000$, YFP-STIM1+CFP-ORAI1, $7.17 \pm 1.19$ pA/pF, $n = 19$; STIM1-YFP+ORAI1-CFP, $7.52 \pm 2.23$ pA/pF, $n = 8$; $p = 0.6792$). b, respective I-V curves from representative cells taken at maximum current density activation. c, fluorescence images from a HEK cell coexpressing ORAI1-CFP and STIM1-YFP (left panel) or YFP-STIM1 (right panel), overlay and calculated FRET image following thapsigargin (TG) application for 7 min. d, time course of HEK cells expressing STIM1 C terminus, ORAI1 and both in combination ($t = 0$, STIM1-C-term(a), $-0.38 \pm 0.07$ pA/pF, $n = 3$; ORAI1(b), $-0.96 \pm 0.19$ pA/pF, $n = 8$; ORAI1+STIM1-C-term(c): $-4.07 \pm 0.49$ pA/pF, $n = 25$; $p_{ac} = 0.0005$, $p_{bc} = 0.00014$). e, respective I-V curves from representative cells taken at $t = 0$ s. f, fluorescence images from a representative cell coexpressing ORAI1-CFP and YFP-STIM1 C terminus, overlay, and FRET images, or STIM1-C terminus alone (lower panel). g, average FRET values determined for the constructs shown in c and f and for ORAI1-CFP + YFP-STIM1 after TG for 7 min. The error bars reflect S.E. The numbers of experiments are given in parentheses. h, left panel, aliquots of ORAI1 (1/5; lower panel; input) used for the binding assay (upper panel). First lane, beads-control + ORAI1; second lane, His-STIM1-C-term + ORAI1. One representative experiment of nine is shown. Right panel, prebound GST-tagged ORAI1 C terminus (first lane) and ORAI1 N terminus (second lane) were probed for their interaction with His$_6$-tagged STIM1 C terminus. One representative experiment of seven is shown. All of the images have scale bars indicating 5 μm.
Dynamic Coupling between STIM1 and ORAI1

and correlated with the multimerization of STIM1 (Fig. 2, c and d). For the latter we employed a FRET based assay (32) utilizing CFP/YFP-tagged STIM1 to monitor STIM1 multimerization that yielded punctuate clusters following thapsigargin application. STIM1 seemed already partially preassociated (32, 33) based on the elevated FRET values (Fig. 2d) and a reciprocal time course that supports the concept of a causal chain of aggregation and association events. The STIM1-ORAI1 interaction seemed to occur on a similar time scale as STIM1 puncta formation (t 1/2 = 15 s, data not shown) in the HEK293 cells. Homomeric ORAI1 interactions as derived from substantial FRET values at resting cell conditions (supplemental Fig. S3) did not change when ER stores were depleted (Fig. 2e). This is in line with the formation of clusters via aggregation of preassociated ORAI1 channel complexes. Furthermore, the dynamic cluster formation of ORAI1-STIM1 is correlated with their interaction as the key event in the activation cascade. Refilling of stores is expected to shut down the ORAI1 activation machinery and was reported (32) to result in a decrease in FRET from STIM1-ORAI1 interaction. Here we show (Fig. 2g) that employing a similar protocol, refilling of ER stores is associated with reversal of FRET between STIM1 and ORAI1, demonstrating disruption of coupling when stores are replenished.

**Dynamic STIM1-ORAI1 Coupling Is Mediated by ORAI1 C Terminus Rather Than Its N Terminus—**In an *in vivo* approach to identify the structural motif(s) within ORAI1 that mediate this coupling to STIM1, we constructed ORAI1 deletion mutants devoid in their whole N or C terminus. Both mutants
showed expression in HEK cells, targeting to the plasma membrane and based on FRET measurements homomeric interactions (data not shown). However, this N- or C-terminal deletion mutant failed to generate store depletion induced Ca\(^{2+}\) entry (data not shown) or currents (Figs. 3 and 4, a and b), suggesting that each of the cytosolic strands is essential for a functional.
activation of ORAI1. A report from Li et al. (34) has shown consistent loss of function of N- and C-terminal ORAI1 deletion mutants but did not examine their interaction with STIM1. To discern whether the lack of these termini on ORAI1 interfered with the dynamic coupling to STIM1, alterations in FRET were monitored in response to ER store depletion. The ΔNterm-ORAI1 was still capable of interacting with STIM1 following thapsigargin application that correlated with its ability to form clusters (Fig. 3, e and g) as observed with ORAI1. In a further approach we analyzed the behavior of the ORAI1 R91W mutant that is linked with severe combined immune deficiency syndrome and leads to the abrogation of CRAC channel function (10). Although ORAI1 R91W mutant accordingly failed to generate currents when coexpressed with STIM1 (Fig. 3, c and d), both proteins exhibited clustered interaction correlating with an increase in FRET following store depletion (Fig. 3, f and g), suggesting a defect in its gating rather than in its coupling to STIM1. Furthermore, the ORAI1 R91W behavior with respect to plasma membrane targeting, homomeric assembly, as well as heteromerization with wild-type ORAI1 did not appear to be different to that of wild-type ORAI1 (supplemental Fig. S2). Despite its defect in generating currents, coexpression of ORAI1 R91W mutant with wild-type ORAI1, expected to yield heteromeric channel complexes, did not reveal in electrophysiological experiments a significant dominant negative effect on current maxima reached, despite a discernable delay in activation (supplemental Fig. S2).

The ORAI1-ΔCterm mutant, however, completely failed to interact with STIM1 concomitant to a lack of observable cluster formation (Fig. 4, e and g). Hence the C terminus of ORAI1 consistent with the in vitro experiments seems to play a dominant role in coupling to STIM1, independent of the N terminus of ORAI1. In accordance, the ORAI1-ΔCterm mutant that is still able to interact with full-length ORAI1 functioned as dominant negative species by inhibiting Ca\(^{2+}\) currents (data not shown), substantiating the essential role of the C terminus in the coupling process with STIM1. A Putative Coiled-coil Domain in ORAI1 C Terminus Mediates Dynamic Coupling to STIM1—The C termini of ORAI1, ORAI2, and ORAI3 proteins include a conserved, putative coiled-coil domain (14, 15), a common structural motif involved in homom- as well as heteromeric protein-protein interactions (35). Based on two different bioinformatic prediction methods applied to ORAI1 coiled-coil motif (amino acids 263–285), point mutation of the central hydrophobic amino acid leucine at position 273 to a hydrophilic serine (ORAI1 L273S) is expected to destabilize coiled-coil structure (36), whereas mutation of leucine 282 terminally located (ORAI1 L282S) is predicted to stabilize coiled-coil formation (supplemental Fig. S1a). Coexpression of this ORAI1 L273S mutant together with STIM1 failed to generate Ca\(^{2+}\) currents (Fig. 4, c and d) as typically seen with wild-type ORAI1. Moreover, this ORAI1 mutant functioned as dominant negative species upon coexpression with full-length ORAI1 (supplemental Fig. S2) in line...
Dynamic Coupling between STIM1 and ORAI1

with a heteromeric interaction and a marked interference with the channel activation process conferred by STIM1. Consistently, FRET imaging revealed (Fig. 4, f and g) a lack of interaction between ORAI1 L273S mutant and STIM1 (t = 8 min: STIM1+ORAI1, 0.093 ± 0.009, n = 8; STIM1+ORAI1 L273S, 0.004 ± 0.005, n = 8, p = 5e-4), together with a lack of cluster formation between the two proteins. The ORAI1 L282S mutant containing the stabilized coiled-coil motif, by contrast, functioned similar to wild-type ORAI1 both in terms of current activation and coupling to STIM1 (supplemental Fig. S1). Consistently, the double mutant ORAI1 L273S L282S showed disrupted communication with STIM1 (supplemental Fig. S1). In conclusion, our results demonstrate a critical role for the putative coiled-coil domain in the C terminus of ORAI1 for its dynamic interaction with STIM1 and the formation of ORAI1-STIM1 clusters. A scheme summarizing the main results of this paper is presented in Fig. 5.

This mechanism is expected to be generally conserved for human ORAI2 and ORAI3 because their C termini similarly contain a putative coiled-coil domain (ORAI2, amino acids 222–254; ORAI3, 266–295) predicted even with higher probability than in ORAI1. It is tempting to speculate that STIM1-ORAI1 coupling involves heteromeric coiled-coil interactions between the respective domains in each C termini with a medium affinity optimized for reversible dissociation of STIM1 from ORAI1. The reported (37) function of ORAI1 as a subunit for the activation of classical transient receptor potential currents might utilize this mechanism as well, to confer responsiveness of classical transient receptor potential channels to store depletion. Transforming the coupling of STIM1 into ORAI1 channel activity apparently involves its N terminus in line with different maximum currents that have been related to distinct ORAI N termini (38, 39) together with arginine at the critical position (Arg91) just at the beginning of the first transmembrane part. The distinct behavior of the ORAI1 R91W and ORAI1 L273S mutants each coexpressed with wild-type ORAI1 (supplemental Fig. S2) is in line with the requirement of fully functional ORAI1 C termini in a putative oligomeric channel complex, whereas a heteromeric complex containing the ORAI1 R91W protein is apparently still able to generate currents.

Our experiments do not exclude that CRAC current activation involves additionally a diffusible messenger as previously suggested and termed calcium influx factor (40). Nonetheless, such a messenger is unlikely involved in the channel activation by the STIM1 C-terminal fragment, because this led to current activation without store depletion. The coupling between the C termini of full-length STIM1 and ORAI1 may occur either directly or indirectly including involvement of auxiliary components (41), when the functional ER approaches the plasma membrane by distances between 10 and 25 nm (30). The recent finding (41) suggesting that ORAI1 is part of a larger macromolecular cluster with an estimated 11–14-nm protrusion to the cytoplasm is compatible with the FRET (<10 nm) observed here between ORAI1 and STIM1 with its large C terminus that is able to extend into the plasma membrane–ER space. Our in vitro pulldown experiments with synthesized proteins of STIM1 C terminus and ORAI1 as well as its N- and C-terminal fragments are consistent with a direct, physical interaction of STIM1 C terminus and ORAI1, but the involvement of additional scaffolding protein(s) may well occur within the cell. The final gating process leading to CRAC current activation requires both cytosolic N and C termini of ORAI1 as well as coupling of STIM1 to the putative C-terminal coiled-coil domain of ORAI1. Further elucidation of components involved in fine tuning of this process will hopefully widen the repertoire for manipulation of this key mechanism by the development of both specific inhibitors as well as activators.

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