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Recent Breakthroughs in the Molecular Mechanism of Capacitative Calcium Entry (With Thoughts on How We Got Here)

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Abstract

Activation of phospholipase C by G-protein-coupled receptors results in release of intracellular Ca^{2+} and activation of Ca^{2+} channels in the plasma membrane. The intracellular release of Ca^{2+} is signaled by the second messenger, inositol 1,4,5-trisphosphate. Ca^{2+} entry involves signaling from depleted intracellular stores to plasma membrane Ca^{2+} channels, a process referred to as *capacitative calcium entry* or *store-operated calcium entry*. The electrophysiological current associated with capacitative calcium entry is the calcium-release-activated calcium current, or I_{crac} . In the twenty years since the inception of the concept of capacitative calcium entry, a variety of activation mechanisms have been proposed, and there has been considerable interest in the possibility of transient receptor potential channels functioning as store-operated channels. However, in the past two years, two major players in both the signaling and permeation mechanisms for store-operated channels have been discovered: Stim1 (and possibly Stim2) and the Orai proteins. Activation of store-operated channels involves an endoplasmic reticulum Ca^{2+} sensor called Stim1. Stim1 acts by redistributing within a small component of the endoplasmic reticulum, approaching the plasma membrane, but does not appear to translocate into the plasma membrane. Stim1, either directly or indirectly, signals to plasma membrane Orai proteins which constitute pore-forming subunits of store-operated channels.

In this special issue of *Cell Calcium*, we highlight major breakthroughs of the past two years that led to the identification of molecules underlying the process of capacitative or store-operated Ca^{2+} entry, specifically Stim1 and Orai1, 2 and/or 3. There has been remarkable concordance in the initial experimental findings with these molecules, and as a result, there will be some degree of overlap in scientific findings and interpretation among different laboratories and thus among the contributions to this issue. In my own laboratory, we also are excited about the clearly established roles of these proteins. In this review, I will summarize some of our findings and also our ideas about some of the more controversial points that have arisen. Initially, however, I would like to summarize what, in my opinion, were the major findings and events that brought us to where we are today.

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The concept of capacitative calcium entry

Most reviews on the topic of capacitative or store-operated entry give major credit to an hypothesis paper I published in *Cell Calcium* in 1986 [1], as well as a subsequent review that clarified some important points [2]. The term “capacitative” calcium entry was originally meant to imply this continuous loading and discharge of a Ca^{2+} store, much as in an electrical circuit, charge must load a capacitor before current can flow through it. In my own mind, this obligatory linkage of release to entry came about from experiments showing reloading of stores through Ca^{2+} entry in parotid and lacrimal acinar cells in two studies published in 1977 [3] and 1978 [4], the latter being the first report to demonstrate refilling of Ca^{2+} stores independently of receptor activation. These two papers concluded that the process of Ca^{2+} entry and release were essentially one and the same, and that entry proceeded through release sites to the cytoplasm. It was not clear where the release sites were at that time, and for the sake of simplicity, we assumed the Ca^{2+} was bound to the plasma membrane itself (Figure1).

The precise pathway by which Ca^{2+} reloaded the internal stores was not initially clear; in 1978 I pointed out that no activation of K^+ ($^{86}\text{Rb}^+$) efflux (an indicator of $[\text{Ca}^{2+}]_i$ changes) was observed [1;4]. Subsequently Casteels and Droogmans [5] reported that refilling occurred without smooth muscle contraction, suggesting either a direct route, or possibly one compartmentalized or protected from the bulk of cytoplasm. This of course turned out to be wrong. With hindsight, it is now clear that the failure of $[\text{Ca}^{2+}]_i$ to rise significantly during refilling is simply due to the buffering speed of endoplasmic reticulum matching or exceeding the rate of Ca^{2+} entry, and possibly (see below) close apposition of plasma membrane channels with endoplasmic reticulum uptake sites. In fact, in some cell types, when the rate of re-filling is pushed to the maximum, transient elevation of $[\text{Ca}^{2+}]_i$ is observed [6]. However, the clearest evidence that Ca^{2+} must enter the cytoplasm directly came from experiments with the sarcoplasmic-endoplasmic reticulum Ca^{2+} ATPase inhibitor, thapsigargin [7], and from the subsequent demonstration of a store-operated Ca^{2+} current by Hoth and Penner [8].

Thapsigargin

Thapsigargin essentially changed capacitative calcium entry from a concept to an experiment. The logic and interpretation of the actions of this drug were much more straightforward than the Ca^{2+} -store-reloading paradigms, and soon after this tool was introduced there was an immediate increase in appreciation and interest in this mode of Ca^{2+} entry. The ability to activate Ca^{2+} entry simply by blocking the intracellular pumps responsible for its sequestration led to a number of important conclusions [7]. First, it was possible to show that entry could be fully activated with no discernible activation of phospholipase C. Second, additivity experiments showed that, at least in the parotid cells that were initially studied, there was no additional pathway activated through phospholipase C-linked muscarinic receptors. Third, the fact that thapsigargin activated Ca^{2+} entry to the same extent as phospholipase C activation, but without increasing inositol trisphosphate (IP_3) demonstrated that passage through the endoplasmic reticulum was not required for access to the cytoplasm; i.e., the permeability of the endoplasmic reticulum membrane to Ca^{2+} was not a determinant of the rate at which Ca^{2+} entered the cytoplasm. This and other arguments favoring a transmembrane flux of Ca^{2+} rather than a transit through intracellular stores were summarized in the *Cell Calcium* review of 1990 [2].

But, perhaps the most significant outcome of the discovery of thapsigargin and other SERCA-inhibiting drugs was that it provided a precise functional and pharmacological definition of capacitative entry. Notably, the ability of thapsigargin to specifically activate

capacitative calcium entry, while minimizing the roles of other upstream players in the pathway (receptors, G-proteins, phospholipase C, etc.) formed the basis for the high throughput assays that led to the discoveries of both Stim and Orai.

I_{crac}

That Ca^{2+} entered the cytoplasm by directly traversing the plasma membrane was unequivocally established by the demonstration of a store-operated or capacitative calcium entry transmembrane current by Hoth and Penner in 1992 [8]. If thapsigargin was responsible for bringing biochemists and cell biologists on board, the demonstration of a calcium-release-activated Ca^{2+} current (I_{crac}) caught the attention of the biophysicists. Hoth and Penner [8;9] provided a thorough biophysical characterization of a very small but highly Ca^{2+} -selective current. This current shared some properties with previously studied Ca^{2+} -selective voltage-activated currents. For example, I_{crac} demonstrated an anomalous mole fraction effect; removing external Ca^{2+} led to loss of current, unless all external divalent cations were removed with chelators, in which case the currents became permeable to monovalent cations, and were actually larger than when conducting Ca^{2+} . However, I_{crac} differed from other known currents in one important way: the single channel conductance was too small to be measured directly, and was subsequently estimated indirectly by noise analysis by Zweifach and Lewis [10] to be of the order of 24 fS (in isotonic Ca^{2+}). This finding suggests that the molecular structure of CRAC channels and the other known Ca^{2+} channels might be very different (which in fact has turned out to be the case). In addition, I_{crac} showed unusual regulation by Ca^{2+} ; intracellular Ca^{2+} inhibited the channels by multiple mechanisms [9;11], while extracellular Ca^{2+} potentiated channel function [12]. These and a number of other findings provided a rigorous fingerprint for the CRAC channel that eventually aided in its correct molecular identification.

Activation mechanisms abound

Numerous reviews have summarized the ideas that have come in and out of vogue in the last twenty years regarding the activation mechanism for capacitative calcium entry [13-19]. Three fundamental mechanisms have been proposed for transmitting the signal from intracellular stores to the plasma membrane: (i) a diffusible message, (ii) conformational coupling [14], and (iii) vesicle secretion [20].

The idea of a diffusible message for capacitative calcium entry was proposed when it was realized that no special path for calcium entry through the endoplasmic reticulum existed, and that the calcium entered the cell directly across the plasma membrane into the cytoplasm [2]. Subsequently, a considerable number of publications have argued for a diffusible signal coupling depletion of intracellular Ca^{2+} stores to calcium entry, and some evidence, albeit more controversial, for the involvement of specific mediators. Two laboratories published findings suggesting an involvement of cyclic GMP [21;22;22], others, arachidonic acid (or one of its metabolites) [23-25]; however, it now seems likely that these mediators act on channels distinct from the store-operated ones [26;27]. The one candidate for a diffusible signal for store-operated channels that has withstood the test of time is one whose structure is not yet known: a Ca^{2+} entry-activating principle partially isolated from store-depleted cells called CIF (for Calcium Influx Factor) [18;28;29]. The evidence for such a messenger, and how it acts within the context of the newly developed Stim1 and Orai story is the subject of another contribution to this issue, and I will not discuss it further here.

Irvine [30] (see also [14]) proposed a conformational coupling model based on the known interaction between plasma membrane dihydropyridine receptors (voltage-dependent calcium channels) and intracellular ryanodine calcium release channels in skeletal muscle. In the phospholipase C signaling system, endoplasmic reticulum IP_3 receptors were

proposed to interact directly with plasma membrane capacitative calcium entry channels. In the case of skeletal muscle, information flows from the t-tubule membrane to the sarcoplasmic reticulum; in the case of the IP₃ receptor and capacitative calcium entry, a fall in luminal Ca²⁺ in the endoplasmic reticulum would induce a conformational change in the IP₃ receptor, and this would be conveyed directly to the plasma membrane channel via a protein-protein interaction. This was an intriguing idea, but there was little direct evidence for it. There is however, evidence for a requirement for close spatial association between endoplasmic reticulum and plasma membrane store-operated channels. Jaconi *et al.* [31] utilized centrifugation to redistribute the organellar contents of oocytes and found that entry only occurred in regions with closely apposed endoplasmic reticulum. Patterson *et al.* [32] used drugs to stimulate peripheral actin polymerization, and disrupted communication between Ca²⁺ stores and plasma membrane store-operated channels (but see [33]).

The TRP story

Hardie and Minke [34;35] first pointed out that the *Drosophila* photoreceptor calcium channel, TRP, was activated downstream of phospholipase C and might be a candidate for a store-operated channel. When mammalian homologs were cloned ([36] the canonical TRPs, or TRPCs), a number of laboratories aggressively pursued this possibility. Early results were encouraging [36-38]. However, it soon became apparent that many of the early findings either resulted either from constitutive activity of over-expressed channels, or were not reproducible in other laboratories [39;40]. Nonetheless, there are numerous reports of diminished store-operated entry following knock-down of TRPC expression (for example, [41], and others reviewed in [19]). Also, it is clear that under some circumstances, TRPC channels can exhibit store-operated activity when ectopically expressed [42-45].

An important issue is that TRPC channels, when experimentally expressed, do not recapitulate the properties of I_{crac} ; rather, they form non-selective cation channels, or at best channels with only modest Ca²⁺ selectivity. Additionally, they have single channel conductances of conventional size, on the order of tens of picosiemens. Thus, if TRPC channels operated as store-operated channels, it would appear that these channels should have characteristics clearly distinct from I_{crac} (for example, [46], others reviewed in [19]). However, while many laboratories have confirmed the basic properties of I_{crac} in hematopoietic cells and other cell types, reports of non- I_{crac} currents and channels have been to now largely restricted to single laboratories. In fact, this can be said in general about published experimental findings implicating TRPC channels in store-operated entry: each of the many encouraging or suggestive results seems restricted to a single laboratory, such that there does not appear to be a consensus among laboratories as to the experimental evidence for store-operated TRPC channels. This is in marked contrast to the finding that, for example, TRPC3, 6 and 7 in their non-store-operated modes can be activated by diacylglycerols, or as is evident from several contributions to this volume, the clear and reproducible store-operated activity of Stim1 and Orai combinations.

Stim1

The role of Stim1 (actually, initially *Drosophila* Stim) in capacitative calcium entry was discovered through a limited RNAi screen of thapsigargin-activated Ca²⁺ entry in *Drosophila* S2 cells by Roos *et al.* [47]. This was followed shortly thereafter by a report from Liou *et al.* [48] who carried out a limited RNAi screen using thapsigargin-activated mammalian HeLa cells. *Drosophila* has a single Stim gene, while mammalian cells have two, designated Stim1 and 2. In the Roos *et al.* study, following the identification of Stim from the S2 screen, they found that knockdown of Stim1, but not Stim2 reduced store-operated entry and I_{crac} in mammalian cells. However, Liou *et al.* reported a slight reduction

in entry by knockdown of Stim2 in HeLa cells. In our laboratory, we found no effect of knockdown of Stim2 in HEK293 cells (unpublished) and no effect of expression of Stim2 in HEK293 cells, even when co-expressed with Orai1 [49]. Soboloff *et al.* [50] reported that Stim2 could act as an inhibitor of store-operated entry; however, this action of Stim2 was only seen when expressed at very high levels. Thus the true physiological function of Stim2 may not yet be known.

It seems clear that the function of Stim1 is to act as the initial sensor of Ca^{2+} levels in the endoplasmic reticulum, or the component of it involved in regulation of I_{crac} . The Ca^{2+} sensing domain is an EF-hand that resides in the lumen of the endoplasmic reticulum, N-terminal to the single transmembrane segment. Several laboratories have demonstrated that mutations in the EF-hand region, presumably reducing Ca^{2+} affinity, result in constitutive activation of Ca^{2+} entry with properties expected of I_{crac} . [48;49;51;52].

Experiments examining the cellular distribution of Stim1 histochemically, or utilizing expression of fluorescent protein fusion constructs have provided intriguing information on the cell biology of this Ca^{2+} sensor. An important finding is that the intracellular distribution of Stim1 does not match well with markers for endoplasmic reticulum (Figure 2). We observed YFP-Stim1 in fibrillar, or more likely tubular structures in HEK293 cells [49], structures not apparent from labeling generic endoplasmic reticulum in the same cell type [53]. It is likely that Stim1 will serve to define a distinct organelle, or more likely a distinct compartment within the endoplasmic reticulum. This is gratifyingly consistent with a number of previous studies which concluded that the pool of Ca^{2+} that regulated store-operated channels appears to be a small component of the total endoplasmic reticulum [54-59].

As interesting as the subcellular localization of Stim1 may be, what is more exciting is the idea that Ca^{2+} dissociation causes the protein to redistribute intracellularly. Liou *et al.* [48] first showed that upon Ca^{2+} store depletion Stim1 appeared to redistribute into punctate structures, and move closer to the plasma membrane. This has been confirmed in a number of laboratories [49;51;52] (Figure 2). However, a major point of controversy remains regarding the localization of Stim1 when it redistributes into punctae. Liou *et al.* reported that Stim1 moved close to the plasma membrane, but using an antibody directed against the YFP on the N-terminus, they failed to detect Stim1 on the plasma membrane [48]. On the other hand, based on surface biotinylation studies, Zhang *et al.* [51] reported that Stim1 actually translocates into the plasma membrane. A third position was taken by Spassova *et al.* [52] who reported that a fraction of Stim1 is present in the plasma membrane where it appears to function in store-operated entry (based on the inhibition of entry by extracellular application of an anti-Stim1 antibody), but they failed to see any change in the amount of plasma membrane Stim1 following Ca^{2+} store depletion.

We repeated the experiment of Liou *et al.* [48] using the same YFP-tagged Stim1 which they kindly provided to us. We confirmed that no YFP-tagged Stim1 could be detected on the plasma membrane of HEK293 cells, either by surface antibody staining and confocal microscopy, or by flow cytometry [49]. On the other hand, Spassova *et al.* [52] clearly observed surface labeling of Stim1 by flow cytometry, using an untagged construct and an antibody directed against the N-terminus. Furthermore, the first report on Stim1, prior to its disclosure as an initiator of capacitative calcium entry, identified it as a surface protein of stromal cells able to interact with B lymphocytes [60], and this surface localization was substantiated in subsequent studies [61;62]. Our interpretation of these disparate findings is that native Stim1 is located in the endoplasmic reticulum and in the plasma membrane, but the addition of YFP to the N-terminus of Stim1 prevents its trafficking to the plasma membrane. This is actually fortuitous, since it allows us to assess the function of Stim1 with

a construct restricted to intracellular sites. With this construct, we [49] confirmed the original observation of Liou *et al.* [48] that the YFP-Stim1 is fully functional, both in rescuing store-operated entry after RNAi knockdown of Stim1, and in generating constitutive entry following mutation of the EF hand domain. A similar finding was reported by Baba *et al.* [63] who found that N-terminally Flag-tagged Stim1 could not be detected in the plasma membrane of a B-cell line, but still was capable of supporting full store-operated entry. In this case, complete knockout of Stim1 was achieved by targeted gene disruption, leaving no doubt that the N-terminally tagged Stim1 could function in the absence of any residual wild-type protein. Finally, as will be discussed subsequently, it is possible to greatly increase store-operated entry by co-expression of Stim1 with Orai1; however, even these very large I_{crac} -like currents were supported by YFP-Stim1, again with no surface expression detected [49]. Our conclusion is that Stim1 does not translocate to the plasma membrane in response to Ca^{2+} store depletion, and plasma membrane Stim1, although likely present, does not play an obligatory role in activating store-operated channels.

Orais

Orai1 was first discovered by Feske *et al.* [64] from a combination of gene mapping from a family with an immunodeficiency attributed to loss of I_{crac} and a whole-genome screen of *Drosophila* S2 cells. Orai1 is located in the plasma membrane and appears to have four transmembrane domains. However, unlike the case for Stim1, which was included in restricted screens based on its signaling domains, Orai1 has no recognizable signaling or channel-like domains and thus required the full genome screen. Soon thereafter, two other groups, also using a similar whole-genome screen of S2 cells, reported on the requirement for Orai (termed CRACM1 by Vig *et al.* [65]) for store-operated entry and for I_{crac} [65;66]. Zhang *et al.* [66] also reported that when *Drosophila* Orai and Stim were co-expressed they appeared to synergize to form unusually large I_{crac} -like currents. This synergy was subsequently reported for mammalian Stim1 and Orai1 by other laboratories [49;67;68]. I_{crac} current densities were increased up to 50 fold by the combination of Stim1 and Orai1, and the resulting currents were indistinguishable from native I_{crac} in both biophysical and pharmacological properties [49;67;68]. This result indicates that these two proteins can fully recapitulate the properties of I_{crac} , a finding notably missing in all previous studies of TRP channels; in fact, it is the distinct pharmacology or selectivity of TRP channels that revealed that TRP channels could function in a store-operated mode in some instances [42-44;69]. This does not necessarily mean that no other players are involved; however, other proteins functioning in a stoichiometric complex with Stim1 and/or Orai1 would have to be constitutively present in considerable excess.

The large I_{crac} -like currents observed with expression of Stim1 and Orai1 immediately led to speculation that Orai1 was likely the CRAC channel itself, or possibly a subunit of it. However, there are no obvious channel pore-like sequences in Orai1. Two laboratories focused on a string of acidic residues near the extracellular boundary of the first transmembrane domain ([70;71] see also [72]). The most interesting mutations targeted a glutamate in position 106 in mammalian Orai1. Mutation to alanine resulted in a nonfunctional channel; however, the conservative mutation of this glutamate to an aspartate (E106D; E180D in *Drosophila* Stim) resulted in a channel with markedly reduced selectivity for Ca^{2+} . This provides strong evidence that this residue functions as part of the Ca^{2+} binding selectivity filter, and indicates that Orai1 is indeed a pore forming subunit of the CRAC channel. Prakriya *et al.* [70] also investigated a glutamate residue at position 190. Mutation of this glutamate to aspartate or even alanine had no effect on channel function; however, the rather extreme alteration to a positively charged glutamine (E190Q) resulted in diminished Ca^{2+} selectivity. Since subtle effects on channel function were seen with only rather drastic changes in the nature of this amino acid, it may be that this mutation alters the

secondary or tertiary structure of the channel, rather than functioning as part of the Ca^{2+} binding site in the channel pore.

In addition to Orai1, mammalian cells also have genes for two additional homologs, Orai2 and Orai3. Unlike the case for Stim2, Orai2 appears to function similarly to Orai1, at least when expressed with Stim1 in HEK293 cells [49]. Orai2 currents are somewhat smaller, however, and it is not yet known if this is an intrinsic property of Orai2 channels, or indicates a lower level of expression in the overexpression studies. Orai3 currents are even smaller, such that Ca^{2+} currents from co-expression of Stim1 and Orai3 in HEK293 cells are below the limits of detection. However, Orai3-dependent currents can be observed when Na^+ carries the current (unpublished observation), and Orai3 can rescue store-operated entry following knockdown of Orai1 in HEK293 cells [49]. Whether Orai2 and 3 form distinct store-operated channels in specific cell types, or function as subunits of heteromeric channels with Orai1 will be a topic of future investigation.

Stim1 – Orai1 Communication

The evidence is very strong that the signaling pathway for capacitative calcium entry begins with the Ca^{2+} sensor, Stim1, and culminates in the activation of channels composed partly or wholly of Orai subunits. The obvious question is how does Stim1 convey information of depleted Ca^{2+} stores to Orai channels? The simplest answer is that when Stim1 coalesces into punctae and approaches the plasma membrane, it directly interacts with Orai channels there. In support of this idea, Yeromin *et al.* [73] reported that *Drosophila* Stim and Orai could be co-immunoprecipitated, and this association was increased by depletion of Ca^{2+} stores. However, Feske *et al.* [64] failed to observe any co-immunoprecipitation of mammalian Stim1 and Orai1. Vig *et al.* [72] reported coimmunoprecipitation of transfected and tagged Stim1 and Orai1, but did not assess effects of store depletion. Nonetheless, it is clear from the work of Luik *et al.* [74] that communication between Stim1 and Orai1 occurs over very short distances; these investigators observed that Orai1 was recruited to sites of Stim1 punctae formation, and that Ca^{2+} entry was spatially restricted to these plasma membrane sites as well. Finally, although a direct interaction between Stim1 and Orai seems the simplest mechanism for signaling from intracellular stores to the plasma membrane channels, there is nothing to rule out the generation of a secondary message downstream of Stim1 that acts on Orai, for example, the CIF and iPLA₂ pathway described by Bolotina in this issue and elsewhere [18].

Summary

In just the past two years, the discovery of Stim and Orai proteins has revolutionized our thinking about capacitative calcium entry and I_{crac} . It is remarkable how quickly different laboratories confirmed the dependence of I_{crac} on these two genes, and confirmed the ability of these two proteins to recapitulate the long-known properties of CRAC channels. In fact, it could be said that these are the only aspects of the I_{crac} story upon which all (or most) seem to agree. By contrast, the roles of TRP channels and other signaling pathways continue to reflect results from one or only a few laboratories, with different groups producing markedly differing findings. The current ideas about Stim1 and Orai action are consistent with a number of earlier ideas about store-operated entry; for example, the presence of a small, specialized component of the endoplasmic reticulum dedicated to communicating with plasma membrane channels; the observation that biophysical properties of CRAC channels differ substantially from conventional ion channels, predicting that they may also differ substantially in their structure; the general finding that Ca^{2+} refills the stores efficiently without resulting in a rise in global cytoplasmic Ca^{2+} and the suggested close physical association between sites of endoplasmic reticulum signaling and plasma membrane Ca^{2+}

entry. One could argue that the original idea of Irvine [30] - conformational coupling - is at least partially vindicated by the current model. However, it appears to be Stim1 rather than IP₃ receptors that are responsible for this coupling. A summary of the store-operated pathway incorporating the functions of Stim1 and Orais is shown in Figure 3. At present there are some general questions that will no doubt be attacked and hopefully resolved in the not too distant future: (i) Are any other players or proteins necessary for activation of capacitative calcium entry and/or I_{crac} ? (ii) What is the role, if any, of plasma membrane Stim1? (iii) What is the composition of native CRAC channels (Orai homo- or heteromultimers; or combinations with TRPs)? (iv) Are there mechanisms of store-operated entry involving other modes of activation (other than Stim1) and other store-operated channels (other than Orais)? In addition, the availability of Stim1 and Orai cDNAs will lead to a detailed structural understanding of the various modes of regulation and activation of this pathway. Such information may ultimately prove of use in designing novel pharmacological reagents to aid in the treatment of a number of diseases in which the store-operated entry pathway is thought to play a role.

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Figure 1. Primitive version of capacitative calcium entry
 The intracellular Ca^{2+} store was envisioned as bound to the plasma membrane, occluding the Ca^{2+} channel. release of the bound Ca^{2+} was associated with gating of the Ca^{2+} channel. Redrawn from [3] with permission.

Figure 2. Stim1 rearranges following Ca^{2+} store depletion, but the endoplasmic reticulum does not

A and B: Thapsigargin causes rearrangement of YFP-Stim1 from a fibrillar or tubular arrangement into punctae. From [49] with permission. C and D: Endoplasmic reticulum-targeted GFP does not redistribute or rearrange following Ca^{2+} store depletion. From [53] with permission.

Figure 3. Current understanding of the roles of Stim1 and Orais

Agonist activation of a plasma membrane receptor (R) results in formation of IP₃, which activates the IP₃ receptor (IP₃R) causing discharge of store Ca²⁺ from a subcompartment of the endoplasmic reticulum. Within this subcompartment, Ca²⁺ binds reversibly to an EF hand motif in Stim1; depletion of Ca²⁺ results in Stim1 without Ca²⁺ bound, which causes Stim1 to redistribute within the endoplasmic reticulum to areas near Orai within the plasma membrane. Stim1 then activates Ca²⁺-selective Orai channels; the mechanism whereby this activation is accomplished is unknown. Stim1 is also present in the plasma membrane, although its function there is unclear.