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New Molecular Players in Capacitative Ca^{2+} Entry

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Summary

Capacitative Ca^{2+} entry links the emptying of intracellular Ca^{2+} stores to the activation of "store-operated" Ca^{2+} channels in the plasma membrane. In the twenty years since the inception of the concept of capacitative Ca^{2+} entry, a number of activation mechanisms have been proposed, and there has been considerable interest in the possibility that TRP channels function 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 the Orai proteins. Stim1 is an endoplasmic reticulum Ca^{2+} sensor. It appears to act 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 signals to plasma membrane Orai proteins, which constitute pore-forming subunits of store-operated channels.

Keywords

Store-operated Ca^{2+} entry; Ca^{2+} channels; Stim1; Orai1; Ca^{2+}-release-activated Ca^{2+} current

Introduction

In a variety of cell types, activation of phospholipase C by G-protein-coupled receptors results in release of intracellular Ca^{2+} stores and activation of Ca^{2+} entry across the plasma membrane. The intracellular release of Ca^{2+} is most commonly signaled by the second messenger, inositol 1,4,5-trisphosphate. Early work on mechanisms of Ca^{2+} mobilization revealed that Ca^{2+} entry involves signaling from depleted intracellular stores to plasma membrane Ca^{2+} channels, a process referred to as *capacitative Ca^{2+} entry* or *store-operated Ca^{2+} entry*. In 2001, my colleagues and I reviewed the state of knowledge about capacitative Ca^{2+} entry for *Journal of Cell Science* emphasizing theories on the activation mechanism and the nature of the channels (Putney, Jr. et al. 2001).

Embarrassingly, while the phenomenology we summarized at that time was correct, almost all of the favored ideas about activation mechanisms and channels have turned out to be either incorrect or, at the very least, incomplete. In just the past two years, major breakthroughs have revealed key players that initiate signaling from depleted stores and form store-operated channels themselves. In this commentary, I provide a snapshot of current knowledge about the new players, Stim1 and the Orai, speculate on how they act, and make some guesses as to where this rapidly moving field is headed. I first, however, briefly review the concept of capacitative Ca^{2+} entry and describe some of the pharmacological and biophysical properties that are important for assessing the actions of the newly discovered components.

Capacitative Ca^{2+} entry

Capacitative Ca^{2+} entry, also called store-operated Ca^{2+} entry, simply refers to a phenomenon in which depletion of intracellular Ca^{2+} stores leads to activation of Ca^{2+}-permeable channels in the plasma membrane. The Ca^{2+} entering through the store-operated channels can then be pumped into the stores, and thus replenish them. Actions of relatively specific inhibitors of sarcoplasmic-endoplasmic reticulum Ca^{2+} (SERCA) pumps (discussed below) and clear localization of structures sensitive to the Ca^{2+}-releasing action of inositol trisphosphate (IP_3) indicate that the intracellular store involved is the endoplasmic reticulum, or a component of it. The function of this pathway is at the very least to maintain sufficient Ca^{2+} in the endoplasmic reticulum, where it is required for proper protein synthesis and folding (Sambrook 1990; Teasdale and Jackson 1996). Indeed, prolonged depletion of endoplasmic Ca^{2+} leads to a stress response and ultimately to apoptosis (Tsukamoto and Kaneko 1993; Bian et al. 1997). The pathway is also needed to support ligand-activated Ca^{2+} signaling, essential for a wide variety of regulated physiological pathways (Berridge et al. 2000).

Thapsigargin

Thapsigargin is a specific inhibitor of SERCA pumps on the endoplasmic reticulum. Its application to cells results in passive depletion of intracellular Ca^{2+} stores and thereby activation of the store-operated channels (Takemura et al. 1989). The ability to activate Ca^{2+} entry simply by blocking these pumps led to a number of important conclusions. First, entry can be fully activated with no discernible activation of phospholipase C. Second, additivity experiments showed that, at least in the parotid cells initially studied, there is no additional pathway activated by phospholipase C-linked receptors. Third, because thapsigargin activates Ca^{2+} entry to the same extent that phospholipase C does, but without increasing IP_3 levels, passage of Ca^{2+} through the endoplasmic reticulum is not required for it to access the cytoplasm (i.e. the permeability of the endoplasmic reticulum membrane to Ca^{2+} does not determine the rate at which Ca^{2+} enters the cytoplasm). This and other arguments favoring a transmembrane flux of Ca^{2+} rather than transit through intracellular stores were summarized in an earlier review (Putney, Jr. 1990).

But, perhaps the most significant outcome of the discovery of thapsigargin and other SERCA-inhibiting drugs was that it provided a functional and pharmacological diagnosis of capacitative entry: a thapsigargin-induced sustained elevation of intracellular Ca^{2+} that is dependent upon extracellular Ca^{2+} is generally attributed to store-operated or capacitative Ca^{2+} entry (although additional pharmacological criteria may sometimes be considered (Putney, Jr. 2001)). Notably, the ability of thapsigargin specifically to activate capacitative Ca^{2+} 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 the functions of both Stim and Orai.

I_{crac}

A decade and a half ago, Hoth and Penner (Hoth and Penner 1992; Hoth and Penner 1993) discovered and characterized a very small but highly Ca^{2+}-selective current, which they termed I_{crac} for *C*a^{2+}-*R*elease-*A*ctivated *C*a^{2+} (CRAC) current. This current was identified earlier by Lewis and Cahalan (Lewis and Cahalan 1989) although it was not recognized as a store-operated current. I_{crac} shares some properties with previously studied Ca^{2+}-selective voltage-activated currents. For example, I_{crac} demonstrates an anomalous mole fraction effect;[1] removing external Ca^{2+} leads to loss of current, unless all external divalent cations are removed by use of chelators, in which case the “CRAC” channels become permeable to monovalent cations, and the current actually becomes larger. However, I_{crac} differs from other known currents in one important way: the single channel conductance is too small to be measured

directly and can only be estimated indirectly by noise analysis (Zweifach and Lewis 1993). The molecular structure of CRAC channels might therefore be very different from those of other known Ca^{2+} channels (this has turned out to be the case). In addition, I_{crac} shows unusual regulation by Ca^{2+}; intracellular Ca^{2+} inhibits CRAC channels by multiple mechanisms (Hoth and Penner 1993; Zweifach and Lewis 1995), whereas extracellular Ca^{2+} potentiates channel function (Zweifach and Lewis 1996). These and a number of other findings have provided a rigorous fingerprint for the CRAC channel that eventually aided 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 signaling mechanism for capacitative Ca^{2+} entry (Putney, Jr. and Bird 1993; Sage et al. 1994; Berridge 1995; Favre et al. 1996; Putney, Jr. 1997; Parekh and Penner 1997; Putney, Jr. and McKay 1999; Barritt 1999; Birnbaumer et al. 2000; Elliott 2001; Gill and Patterson 2004; Parekh and Putney, Jr. 2005; Bolotina and Csutora 2005; Rosado et al. 2005). Two fundamental mechanisms have been proposed for transmitting the signal from intracellular stores to the plasma membrane: (i) a diffusible message (Randriamampita and Tsien 1993; Bolotina and Csutora 2005) and (ii) conformational coupling of proteins in the intracellular store membranes to plasma membrane channels (Irvine 1990; Berridge 1995). It has also been suggested that the activation mechanism might involve the insertion of active channels into the plasma membrane vesicle secretion ((Somasundaram et al. 1995; Yao et al. 1999), but see (Scott et al. 2003)). However, this idea does not address the more fundamental question of how the signaling is initiated from Ca^{2+} depleted stores. This concept is not discussed further here.

Early ideas about capacitative Ca^{2+} entry envisioned specialized structures permitting direct access of external Ca^{2+} to the intracellular stores (Putney, Jr. 1977; Casteels and Droogmans 1981). When it was later realized the Ca^{2+} entered the cell directly across the plasma membrane into the cytoplasm, there was no apparent connection between the stores and the channels leading to the idea that a diffusible signal might be involved (Putney, Jr. 1990). However, there is precedent for direct plasma membrane – sarcoplasmic reticulum communication in the case of the very specialized skeletal muscle cells. In the process of excitation – contraction coupling in skeletal muscle, plasma membrane dihydropyridine receptors (voltage-dependent Ca^{2+} channels) interact with, and directly activated intracellular ryanodine Ca^{2+} release channels. This led Irvine ((Irvine 1990) (see also (Berridge 1995)) to propose an analagous conformational coupling model to explain regulated Ca^{2+} entry in cells that utilize the phospholipase C - IP_3 signaling mechanism: endoplasmic reticulum IP_3 receptors would interact directly with plasma membrane capacitative Ca^{2+} entry channels. In the case of skeletal muscle, information flows from the t-tubule membrane to the sarcoplasmic reticulum; in the case of the IP_3 receptor and capacitative Ca^{2+} entry, a fall in luminal Ca^{2+} in the endoplasmic reticulum was proposed to induce a conformational change in the IP_3 receptor, and this would be conveyed directly to the plasma membrane channel by 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

[1]Anomolous mole fraction effect refers to a phenomenon whereby a mixture of two permeant species produces a current less than that obtained with either component of mixture alone (Almers and McCleskey 1984). In the case of Ca^{2+} channels, the most striking instance of such behavior is seen with divalent cation-selective channels that pass essentially no monovalent cations unless the concentration of divalent cations is very low, generally in the low μM range or less. The favored interpretation is that when divalent cations are present, they bind to and reside for relatively long times on a site within the channel known as a selectivity filter (Hess and Tsien 1984). Ions are thought to permeate such channels in a single-file manner, the rate for a particular species depending both on the permeability of the pore for that species as well as the affinity or off-rate from the selectivity filter. Thus, when Ca^{2+} concentration is reduced below its affinity for the selectivity filter, the site is no longer occupied for the majority of the time, and more permeable Na^{+} ions can freely permeate.

membrane store-operated channels. Jaconi et al. (Jaconi et al. 1997) 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. (Patterson et al. 1999) used drugs to stimulate peripheral actin polymerization, and disrupted communication between Ca^{2+} stores and plasma membrane store-operated channels (but see (Bakowski et al. 2001)). Golovina et al. (Golovina 2005) demonstrated that in astrocytes, store-operated Ca^{2+} entry occurred at sites of close plasma membrane – endoplasmic reticulum apposition.

Numerous publications have argued that a diffusible signal couples depletion of intracellular Ca^{2+} stores to Ca^{2+} entry, and some evidence, albeit more controversial, supports the involvement of specific mediators. Two laboratories published findings suggesting cyclic GMP is involved (Pandol and Schoeffield-Payne 1990; Xu et al. 1994); others have implicated arachidonic acid (or one of its metabolites) (Graier et al. 1995; Gailly 1998; Rzigalinski et al. 1999). However, it now seems likely that these mediators act on channels distinct from the store-operated ones (Thompson 1997; Mignen et al. 2005). 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-activator partially purified from store-depleted cells called CIF (for Ca^{2+} Influx Factor) (Randriamampita and Tsien 1993; Thomas and Hanley 1995; Bolotina and Csutora 2005). A role for such a messenger within the context of the new Stim1 and Orai story is certainly well within the realm of possibility.

The TRP story

Hardie and Minke (Hardie and Minke 1992; Hardie and Minke 1993) first pointed out that the *Drosophila* photoreceptor Ca^{2+} channel, TRP, is activated downstream of phospholipase C and thus is a candidate for a store-operated channel. When its closest mammalian relatives were cloned (the seven canonical TRPs, or TRPCs), a number of laboratories aggressively pursued this possibility. Early results were encouraging (Zhu et al. 1996; Zitt et al. 1996; Kiselyov et al. 1998). However, it soon became apparent that many of the early findings either resulted from misinterpretation of constitutive activity of over-expressed channels, or could not be reproduced in other laboratories (Zhu et al. 1998; Trebak et al. 2003). Also, subsequent work on the *Drosophila* TRP channel demonstrated that this is clearly not a store-operated channel (Acharya et al. 1997). Nonetheless, there are numerous reports of diminished store-operated entry when TRPC expression is supressed or eliminated (for example, (Mori et al. 2002), and others reviewed in (Parekh and Putney, Jr. 2005)). Also, it is clear that, under some circumstances, TRPC channels can exhibit what appears to be store-operated activity when ectopically expressed (Vazquez et al. 2001; Vazquez et al. 2003; Lievremont et al. 2004; Zeng et al. 2004). The major problem is that when experimentally introduced into cells, TRPC channels do not recapitulate the properties of I_{crac}; rather, they form non-selective cation channels, or, at best, channels that show only modest Ca^{2+} selectivity. More significantly, they have single channel conductances of conventional size (tens of picosiemens). A number of laboratories have demonstrated that TRPC channels can function physiologically as non-store-operated channels, activated downstream of phospholipase C, like the original *Drosophila* channel (Putney, Jr. 2005; Dietrich et al. 2005). This does not, however, rule out the possibility that TRPCs might also function as store-operated channels when expressed in different contexts, for example, when assembled in signaling complexes of differing composition. There are a number of examples of store-operated Ca^{2+} entry with properties distinct from I_{crac} (for example, (Krause et al. 1996; Trepakova et al. 2000), others reviewed in (Parekh and Putney, Jr. 2005)), and perhaps TRPCs function as store-operated channels in such instances.

Stim1

Almost simultaneously, two laboratories discovered the role of Stim1 (actually, initially *Drosophila* Stim) in capacitative Ca^{2+} entry by use of limited RNAi screens of thapsigargin-activated Ca^{2+} entry in *Drosophila* S2 cells (Roos et al. 2005) or in mammalian HeLa cells (Liou et al. 2005). *Drosophila* has a single Stim gene, whereas mammals have two, Stim1 and Stim2. Roos et al. found that knocking down Stim1, but not Stim2, reduces store-operated entry and I_{crac} in mammalian cells. However, Liou et al. reported a slight reduction in entry in HeLa cells by knocking down Stim2 as well. We found no effect of knockdown of Stim2 in HEK293 cells (Putney, J.W., Jr. et al., unpublished) and no effect of expression of Stim2 in HEK293 cells, even when co-expressed with Orai1 (Mercer et al. 2006). Soboloff et al. (Soboloff et al. 2006a) reported that Stim2 can act as an inhibitor of store-operated entry; however, this action of Stim2 is only seen when it is expressed at very high levels. Thus the true physiological function of Stim2 requires further investigation.

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 is N-terminal to the single transmembrane segment, and oriented in the lumen of the endoplasmic reticulum. Several laboratories have demonstrated that mutations in the EF-hand region, which presumably reduce Ca^{2+} affinity, result in constitutive activation of store-operated entry and I_{crac}. (Liou et al. 2005; Zhang et al. 2005; Spassova et al. 2006; Mercer et al. 2006).

Experiments examining the cellular distribution of Stim1 by histochemistry or fluorescently tagged Stim1 with real-time imaging techniques have provided intriguing information on the cell biology of this Ca^{2+} sensor. Liou et al. (Liou et al. 2005) reported that Stim1 appeared to be located in the endoplasmic reticulum. However, we observed Stim1 with enhanced yellow fluorescent protein fused to its N-terminus (EYFP-Stim1) in fibrillar, or possibly tubular patterns in HEK293 cells (Mercer et al. 2006); these patterns are not apparent from labeling of generic endoplasmic reticulum in the same cell type (Ribeiro et al. 2000). A similar conclusion was reached by Baba et al. (Baba et al. 2006) who imaged Stim1 and endoplasmic reticulum markers within the same cell. In that study, the unique distribution of Stim1 was apparently dependent on the microtubular cytoskeleton (Baba et al. 2006). Thus, Stim1 may reside on a distinct organelle, or more likely a distinct compartment within the endoplasmic reticulum. This would be consistent with a number of previous studies that concluded that the pool of Ca^{2+} that regulates store-operated channels is a small component of the total endoplasmic reticulum (Ribeiro and Putney, Jr. 1996; Parekh et al. 1997; Huang and Putney, Jr. 1998; Broad et al. 1999; Turner et al. 2003; Wisnoskey et al. 2003).

An even more exciting finding is that Ca^{2+} dissociation causes Stim1 to redistribute into punctate structures, and move closer to the plasma membrane (Liou et al. 2005; Zhang et al. 2005; Spassova et al. 2006; Mercer et al. 2006). However, a major point of controversy remains the subcellular localization of Stim1 when it redistributes into punctae. Liou et al. reported that EYFP-Stim1 moved close to the plasma membrane, but using an antibody directed against the EYFP on the N-terminus, they failed to detect it on the plasma membrane (Liou et al. 2005). In contrast, on the basis of surface biotinylation studies, Zhang et al. (Zhang et al. 2005) reported that Stim1 actually translocates into the plasma membrane. A third position was taken by Spassova et al. (Spassova et al. 2006). They 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.

Mercer et al. (Mercer et al. 2006) repeated the experiments performed by Liou et al. (Liou et al. 2005), using the same EYFP-tagged Stim1. These authors confirmed that no EYFP-tagged Stim1 can be detected on the plasma membrane of HEK293 cells, by surface antibody staining and confocal microscopy or by flow cytometry (Mercer et al. 2006). Xu et al. (Xu et al. 2006) reached a similar conclusion, on the basis of studies with a Stim1 N-terminally tagged with a pH sensitive fluorescent indicator. Likewise, Wu et al. (Wu et al. 2006) failed to observe plasma membrane Stim1 by immunoelectronmicroscopy, by utilizing an N-terminally horesradish peroxidase-tagged Stim1. However, Spassova et al. (Spassova et al. 2006) 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 Ca^{2+} entry, identified it as a surface protein on stromal cells involved in their interaction with B lymphocytes (Oritani and Kincade 1996), and this surface localization was substantiated in subsequent studies (Manji et al. 2000; Williams et al. 2002).

A recent report by (Hauser and Tsien 2007) provides and explanation for these disparate findings: the large additions, such as EYFP, 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. EYFP-Stim1 is fully functional, both rescuing store-operated entry after RNAi-mediated knockdown of Stim1, and generating constitutive entry following mutation of the EF hand domain (Liou et al. 2005; Mercer et al. 2006). Similarly, Baba et al. (Baba et al. 2006) found that N-terminally Flag-tagged Stim1 could not be detected in the plasma membrane of a B-cell line, but still fully supports store-operated Ca^{2+} entry. In this case, they knocked out Stim1 by targeted gene disruption, leaving no doubt that the N-terminally tagged Stim1 can function in the absence of any residual wild-type protein. As I discuss below, co-expression of Stim1 with Orai1greatly increases store-operated entry; however, even these very large I_{crac}-like currents are supported by EYFP-Stim1, and again no surface expression can be detected (Mercer et al. 2006). Finally, Huang et al. (Huang et al. 2006) demonstrated that the cytoplasmic C-terminal region of Stim1, expressed in the cytoplasm, was sufficient to activate store-operated entry. Clearly this construct cannot produce any plasma membrane Stim1 with proper orientation. My conclusion is that Stim1 does not translocate to the plasma membrane in response to Ca^{2+} store depletion, and that plasma membrane Stim1, although probably present, does not play an obligatory role in activating store-operated channels.

Orai

Orai1 was first reported (and named) by Feske et al. (Feske et al. 2006) through a combination of gene mapping in a family with an immunodeficiency attributed to loss of I_{crac} and a whole-genome screen of *Drosophila* S2 cells. Almost simultaneously, two other groups, also using a similar whole-genome screen of S2 cells, reported that Orai (termed CRACM1 by Vig et al. (Vig et al. 2006a)) is essential for store-operated entry and for I_{crac} (Zhang et al. 2006; Vig et al. 2006a). Orai1 is located in the plasma membrane and appears to have four transmembrane domains. 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 for its detection. When Orai and Stim are co-expressed they synergize to generate unusually large I_{crac}-like currents (Zhang et al. 2006; Mercer et al. 2006; Peinelt et al. 2006; Soboloff et al. 2006b) which are indistinguishable from native I_{crac} in both biophysical and pharmacological properties (Zhang et al. 2006; Mercer et al. 2006; Peinelt et al. 2006; Soboloff et al. 2006b). These two proteins can thus fully recapitulate the properties of I_{crac},. 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 upon co-expression of Stim1 and Orai1 immediately led to speculation that Orai1 is the CRAC channel itself or a subunit of it. However, there are no obvious channel pore-like sequences in Orai1. Three laboratories focused on a string of acidic residues near the extracellular boundary of the first transmembrane domain (Prakriya et al. 2006; Yeromin et al. 2006; Vig et al. 2006b). The most interesting mutations affect glutamate 106 in mammalian Orai1, and the corresponding residue in *Drosophila* Orai. Mutation to alanine results in a non-functional channel; however, mutation to an aspartate (E106D; E180D in *Drosophila* Stim) results in a functional channel that has markedly reduced selectivity for Ca^{2+}. Acidic residues just downstream from E180 in *Drosophila* Orai confer the well-known high sensitivity of I_{crac} to Gd^{3+} (Yeromin et al. 2006). These findings provide strong evidence that this region functions as part of the Ca^{2+} selectivity filter and indicate that Orai1 is indeed a pore-forming subunit of the CRAC channel.

Prakriya et al. (Prakriya et al. 2006) and Vig et al. (Vig et al. 2006b) have also investigated a glutamate residue at position 190. Mutation of this residue to aspartate or even alanine had no effect on channel function; however, alteration to glutamine (E190Q) resulted in diminished Ca^{2+} selectivity. Since the complete loss of charge from this amino acid (the mutation to alanine) had no effect on channel selectivity, it may be that the mutation to glutamine alters the secondary or tertiary structure of the channel, rather than indicating a function as part of the Ca^{2+} binding site in the channel pore.

Mammalian cells have two additional proteins, Orai2 and Orai3. Orai2 appears to function similarly to Orai1, at least when expressed with Stim1 in HEK293 cells (Mercer et al. 2006). The Orai2 Ca^{2+} currents were somewhat smaller, however, and whether this is an intrinsic property of Orai2 channels, or indicates a lower level of expression in this particular study is not known. Orai3 Ca^{2+} currents were even smaller; those resulting from co-expression of Stim1 and Orai3 in HEK293 cells were 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 when Orai1 is knocked down in HEK293 cells (Mercer et al. 2006). 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 Ca^{2+} 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. (Yeromin et al. 2006) reported that *Drosophila* Stim and Orai can be co-immunoprecipitated, and this association is increased by depletion of Ca^{2+} stores. However, Feske et al. (Feske et al. 2006) failed to observe any co-immumoprecipitation of mammalian Stim1 and Orai1. Vig et al. reported co-immunoprecipitation of transfected and tagged Stim1 and Orai1 but did not assess effects of store depletion (Vig et al. 2006b). Nonetheless, it is clear from the work of Luik et al. (Luik et al. 2006)and Xu et al. (Xu et al. 2006) that communication between Stim1 and Orai1 occurs over very short distances. These investigators observed that Orai1 is recruited to sites of Stim1 punctae formation, and Luik et al. (Luik et al. 2006) showed that Ca^{2+} entry is spatially restricted to these plasma membrane sites as well. Wu et al. (Wu et al. 2006) estimated that Stim1 localizes in junctional endoplasmic reticulum within 10–25 nm of the plasma membrane. 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 proposed CIF and $iPLA_2$ pathway (Bolotina and Csutora 2005).

Conclusions

In just the past two years, the discoveries of Stim and Orai proteins have revolutionized our thinking about capacitative Ca^{2+} entry and I_{crac}. It is remarkable how quickly different laboratories confirmed the dependence of I_{crac} on these two proteins, and confirmed their ability 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. The current findings with Stim1 and Orai action are consistent with a number of earlier ideas about store-operated entry. Some of these earlier ideas are: 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, which indicated that they might also differ substantially in their structure; the general finding that Ca^{2+} refills the stores efficiently without causing 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 might argue that Irvine's original idea (Irvine 1990) of conformational coupling is at least partially vindicated by the current model. However, it appears to be Stim1 rather than IP_3 receptors that are responsible for this coupling.

Figure 1 summarizes the store-operated signaling pathway, incorporating the roles of Stim1 and Orai. Several general questions remain. (i) Are any other players or proteins necessary for activation of capacitative Ca^{2+} 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 – are these Orai homo- or heteromultimers; or combinations with TRPs? (iv) Are there mechanisms of store-operated entry involving other modes of activation and other store-operated channels? 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. The development of animal models will lead to a better understanding of the roles of these proteins in specific physiological and pathological processes. Such information may ultimately prove useful in design of 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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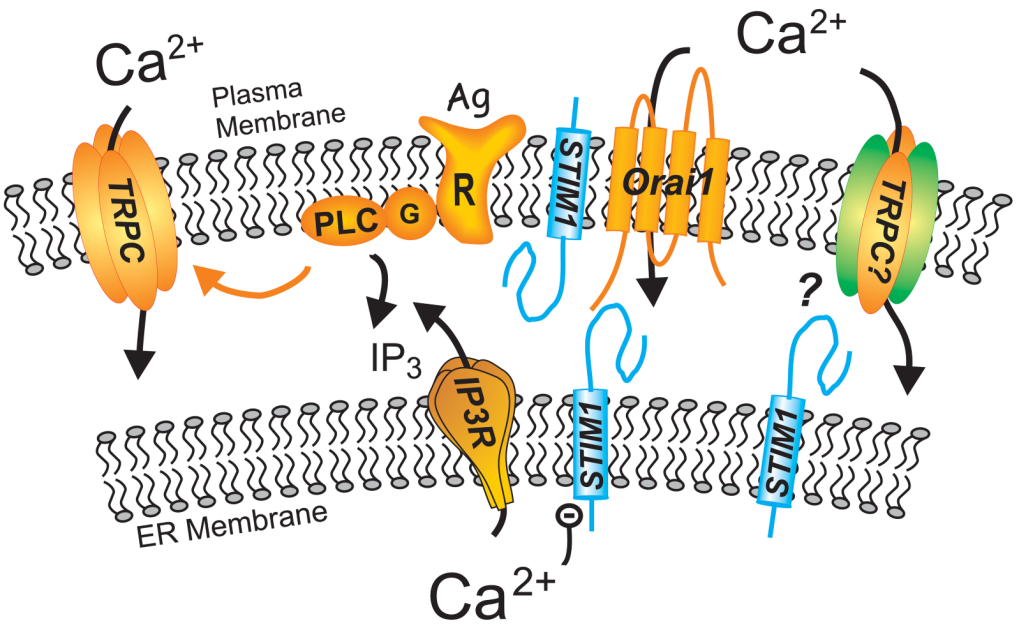

Figure 1. Current understanding of the roles of Stim1 and Orai

Agonist (Ag) activation of a plasma membrane receptor (R) results in formation of IP_3, which activates the IP_3 receptor (IP3R) causing discharge of stored Ca^{2+} from a subcompartment of the endoplasmic reticulum. Within this subcompartment, Ca^{2+} binds reversibly to an EF hand motif in Stim1; depletion of Ca^{2+} results in Ca^{2+} dissociation from Stim1, which causes Stim1 to redistribute within the endoplasmic reticulum to areas near Orai channels that reside in the plasma membrane. Stim1 then activates Ca^{2+}-selective Orai channels; the mechanism by which this activation is accomplished is unknown at present. Stim1 is also present in the plasma membrane, although its function there is unclear. TRPC channels can also be activated by phospholipase C (PLC) –dependent mechanisms. There is evidence that in some instances, perhaps when combined with other subunits, they can function as store-operated channels, perhaps also involving Stim1 as an activator.