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Review

# Emerging perspectives in store-operated $Ca^{2+}$ entry: Roles of Orai, Stim and TRP

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**Abstract**

Depletion of intracellular $Ca^{2+}$ stores induces $Ca^{2+}$ influx across the plasma membrane through store-operated channels (SOCs). This store-operated $Ca^{2+}$ influx is important for the replenishment of the $Ca^{2+}$ stores, and is also involved in many signaling processes by virtue of the ability of intracellular $Ca^{2+}$ to act as a second messenger. For many years, the molecular identities of particular SOCs, as well as the signaling mechanisms by which these channels are activated, have been elusive. Recently, however, the mammalian proteins STIM1 and Orai1 were shown to be necessary for the activation of store-operated $Ca^{2+}$ entry in a variety of mammalian cells. Here we present molecular, pharmacological, and electrophysiological properties of SOCs, with particular focus on the roles that STIM1 and Orai1 may play in the signaling processes that regulate various pathways of store-operated entry.
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$Ca^{2+}$ is an essential second messenger in virtually all cell types, and it regulates a wide spectrum of events from neurotransmission to gene transcription. Cytoplasmic $Ca^{2+}$ can be elevated in cells either by release from internal stores located in the endoplasmic reticulum (ER) or by entry across the plasma membrane [9]. In many cases, intracellular $Ca^{2+}$ release and $Ca^{2+}$ entry occur in concert with one another, and thus both routes of increasing the intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) are often necessary for proper signaling to occur.

A major mechanism of $Ca^{2+}$ release involves the phosphoinositide (PI) pathway, whereby plasma membrane receptors of the G-protein-linked or tyrosine kinase families activate specific phospholipase C (PLC) isoforms, resulting in the cleavage of phosphatidylinositol 4,5-bisphosphate ($PIP_2$) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate ($IP_3$). DAG is most recognizable as an activator of protein kinase C (PKC), but can also play a more direct role in regulating $Ca^{2+}$ signal generation. $IP_3$ is a critical $Ca^{2+}$ releasing agent in the cell, and it achieves this by activating the $IP_3$ receptor ($IP_3R$) located in the ER membrane; binding of $IP_3$ to its receptor results in the receptor/channel opening and release of $Ca^{2+}$ from the ER into the cytoplasm [7].

Activation of the PI pathway also results in $Ca^{2+}$ entry by 2 important mechanisms. First, second messengers generated downstream of activation of the PI pathway, such as DAG, arachidonic acid (AA), and $Ca^{2+}$ itself [11], can directly activate $Ca^{2+}$ entry channels in the plasma membrane; these channels

*Abbreviations:* $[Ca^{2+}]_i$, intracellular $Ca^{2+}$ concentration; 2-APB, 2-aminoethoxydiphenylborane; AA, arachidonic acid; ARC, arachidonate-regulated channel; CCE, capacitative $Ca^{2+}$ entry; CIF, $Ca^2$ influx factor; DAG, diacylglycerol; ER, endoplasmic reticulum; EYFP, enhanced yellow fluorescent protein; $I_{CRAC}$, $Ca^{2+}$ release-activated $Ca^{2+}$ current; $IP_3$, inositol 1,4,5-trisphosphate; $IP_3R$, inositol 1,4,5-trisphosphate receptor; OAG, 1-oleoyl-2-acetyl-*sn*-glycerol; PI, phosphoinositide; $PIP_2$, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; $PLA_2$, phospholipase $A_2$; PLC, phospholipase C; ROC, receptor-operated channel; SAM, sterile-alpha motif; SCID, severe combined immunodeficiency; SERCA, sarco/endoplasmic reticulum $Ca^{2+}$-ATPase; SOC, store-operated channel; TRP, transient receptor potential

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are referred to as receptor-operated channels (ROCs). Second, the release of $Ca^{2+}$ from the ER induced by $IP_3$ leaves the ER in a state of $Ca^{2+}$ depletion; this depletion of ER $Ca^{2+}$ activates plasma membrane $Ca^{2+}$ entry [98], a process originally termed capacitative calcium entry (CCE) [128], through channels generally referred to as store-operated channels (SOCs). This review will focus on $Ca^{2+}$ entry through SOCs, covering the most recent findings in the activation mechanisms of SOCs, the pharmacological, electrophysiological, and molecular properties of SOCs, and physiological and pathophysiological roles for SOCs. For more information on other modes of receptor-regulated entry, the reader is referred to recent reviews [11,140].

## 1. Overview of store-operated entry

By definition, SOCs are activated exclusively as a result of depletion of intracellular $Ca^{2+}$ stores [86]. Experimentally, entry through SOCs can be distinguished from other modes of $Ca^{2+}$ entry by employing protocols that achieve ER $Ca^{2+}$ depletion independently of activation of other signaling pathways. Paramount to the idea that SOC activation relies exclusively on store depletion is the fact that the ER must be able to communicate $Ca^{2+}$ store depletion to SOCs within the plasma membrane in some way.

The existence of SOCs was first postulated in 1986 [98]. Since then, the molecular nature of the process, i.e., the channels responsible for the $Ca^{2+}$ influx and the signaling mechanisms that result in activation of the channels, has remained enigmatic. It is important to note that store-operated entry does not necessarily refer to a single mechanism of $Ca^{2+}$ entry, nor does SOC necessarily refer to a single $Ca^{2+}$ entry channel; instead, any channel that can be shown to exhibit $Ca^{2+}$ store-dependent activity can be referred to as a SOC. The most studied and best characterized SOC current is the $Ca^{2+}$ release-activated $Ca^{2+}$ current, or $I_{CRAC}$, which was first characterized in mast cells [45,46], but has been measured in a variety of cells ranging from T and B-lymphocytes to hepatocytes [85]. $I_{CRAC}$ is a non-voltage-gated current, and is notable for its very high selectivity for $Ca^{2+}$, as indicated by its strong inward rectification, highly positive reversal potential, and low conductance [85,86,97]; other store-operated entry currents that have been characterized generally exhibit less stringent ionic selectivities and a variety of conductances [85,86]. The mechanism by which $I_{CRAC}$ is activated, including the molecular identity of the CRAC channel, has long been the most sought-after signaling paradigm in the store-operated entry field. For some time now, a significant effort has been made to determine whether or not members of the transient receptor potential (TRP) family of ion channels are molecular components of the CRAC channel. Particular focus has concentrated on the canonical TRPs (TRPCs), since it has been shown that these ion channels are activated downstream of PLC activation and, in some cases, can operate as SOCs (see Section 5.1); however, as of yet, no TRPC channel has passed the ultimate test—that is, the ability to fully recapitulate the electrophysiological and pharmacological properties of $I_{CRAC}$. Nevertheless, it is important to note that TRP channels may function in certain physiological contexts as SOCs, despite the fact that this activity is distinct from $I_{CRAC}$. Now, the quest to uncover the molecular identity of the $I_{CRAC}$ channel and its mechanism of activation has come to an important new turning point with the recent discoveries that two proteins, STIM1 and Orai1, play obligatory roles in the activation of $I_{CRAC}$. As will be discussed in Section 4, accumulating evidence indicates that STIM1 acts as a sensor of ER store content, while Orai1 may be the $I_{CRAC}$ channel itself.

Regardless of its form, whether it be via $I_{CRAC}$ or another SOC, store-operated $Ca^{2+}$ influx accomplishes several critical functions within the cell. First, $Ca^{2+}$ that enters the cell via the SOC pathway replenishes the ER $Ca^{2+}$ stores following a release event, thus maintaining the ability of the ER to release $Ca^{2+}$ into the cytoplasm in response to subsequent $Ca^{2+}$ releasing stimuli. This is most notable in cells that respond to activation of the PI pathway with $[Ca^{2+}]$i oscillations, since ablation of $Ca^{2+}$ entry by removing extracellular $Ca^{2+}$ or by other pharmacological or molecular means will often times preclude a cell's ability to maintain $[Ca^{2+}]_i$ oscillations (see Section 6). Second, $Ca^{2+}$ concentrations within the ER must be maintained at sufficient levels in order for the organelle to carry out many of its fundamental functions. Thus, chronic depletion of ER $Ca^{2+}$, as would occur in the absence of store-operated $Ca^{2+}$ entry, can influence ER-dependent processes such as protein folding and trafficking, the ER stress response, and apoptosis [20]. And third, it should be noted that $Ca^{2+}$ that enters the cell via the store-operated pathway first accesses the cytoplasm before entering the ER and, in many cases, results in sustained elevation in $[Ca^{2+}]_i$ levels. This is significant in that for some signaling events, in particular T-lymphocyte activation, it is the increase in cytoplasmic $Ca^{2+}$ that results from store-operated entry, as opposed to that which results from $Ca^{2+}$ released from the ER, that is responsible for signaling [54]. Thus, store-operated entry can influence many aspects of cell biology, and it is vital that we understand the molecular compositions of all forms of store-operated entry so that we may fully appreciate its contribution to normal physiological as well as pathophysiological states of human health.

## 2. Pharmacology of store-operated entry

The use of pharmacological inhibitors of sarco/endoplasmic reticulum $Ca^{2+}$-ATPase (SERCA) pumps, which are responsible for pumping $Ca^{2+}$ into the lumen of the ER from the cytoplasm, has been invaluable in the study of SOC function and regulation. These compounds, which include thapsigargin, cyclopiazonic acid and di-*tert*-butylhydroquinone, achieve $Ca^{2+}$ store depletion by allowing a constitutive passive leak of $Ca^{2+}$ from the ER to occur unchecked due to the inability of the inhibited SERCA pumps to replenish the ER [21]. Further, ER depletion can be effected by dialysis of cells with high concentrations of $Ca^{2+}$ buffers such as EGTA or BAPTA, which effectively reduce the free $Ca^{2+}$ concentration in the cytosol available to the SERCA pumps. Finally, direct buffering of ER $Ca^{2+}$ with membrane permeant *N,N,N′,N′*-tetrakis(2-pyridylmethyl)ethylene diamine (TPEN) reduces the free $Ca^{2+}$ concentration in the ER and activates store-operated entry. Hence, the pharmacological tools

with which SOC activation can be revealed are quite extensive; however, these tools do not allow for manipulation of the entry process itself since they act on mechanisms that are upstream of channel activation. In order to define roles for store-operated entry in physiological processes, it is important to have available tools by which one can predictably and specifically antagonize the entry process. Unfortunately, the SOC field has chronically been plagued by a lack of pharmacological inhibitors that specifically target store-operated $Ca^{2+}$ influx.

Currently available pharmacological inhibitors of the store-operated entry pathway lack optimal specificity. Indeed, many agents such as econazole and SK&F-96365 that are known to inhibit SOCs were also noted to inhibit other ion channels, particularly voltage-dependent channels, within the same concentration ranges [34,99]. Another drug called 2-aminoethoxydiphenylborane (2-APB) described originally as an $IP_3R$ antagonist [69], was shown later to be a direct inhibitor of SOCs, having no effect on voltage-dependent $Ca^{2+}$ entry channels or arachidonic acid-activated $Ca^{2+}$ channels [2,15,96]. However, 2-APB was later shown to interfere with a wide variety of transport mechanisms including SERCA pumps, potassium channels and mitochondrial $Ca^{2+}$ efflux (for review see [86]). In addition, 2-APB has been found to activate TRPV1, TRPV2 and TRPV3, members of the heat-gated vanilloid TRP (TRPV) family of cation channels [23,47]. Despite these various effects of 2-APB unrelated to store-operated entry, the drug remains an important tool in the study of $Ca^{2+}$ influx. Store-operated entry and $I_{CRAC}$ in particular have the unique characteristics of being potentiated by low concentrations of 2-APB (1–5 μM), whereas they are inhibited by concentrations greater than 10 μM [66,96]. And although the mechanism by which 2-APB exerts these effects on SOCs is unknown, this biphasic sensitivity to 2-APB is often used as a "fingerprint" that helps define the store-operated nature of a particular $Ca^{2+}$ influx process.

There are other reagents that block store-operated entry but also block voltage-dependent $Ca^{2+}$ entry channels with equal efficiencies. These drugs include L561582 [29,99], SC38249 [25,99], LU52396 [24,99] and tetrandine [53,99]. Other drugs such as cytochrome P-450 inhibitors or reagents that inhibit enzymes of arachidonic acid metabolism (cyclooxygenase and lipoxygenase inhibitors) are also known to inhibit store-operated entry (for review see [99]). However, these drugs are not specific and their exact action on store-operated entry is not clear.

The most reliable inhibitors of SOCs remain trivalents such as $La^{3+}$ and $Gd^{3+}$. The latter when used at concentrations in the low micromolar (1–5 μM) range has proven to be a specific blocker of store-operated entry in many cell types without affecting arachidonic acid-activated $Ca^{2+}$ entry or the $Ca^{2+}$ entry pathway mediated by receptor-activated TRPC channels [17,63,135]. However, this inorganic inhibitor precipitates with divalent anions and thus cannot be used *in vivo*. Its use is therefore restricted to *in vitro* studies using cultured cells in media with controlled compositions.

The quest for specific inhibitors of the various SOCs expressed in human cells is clearly in its infancy and new drugs that interfere with SOC activation are likely to be promising tools for treatment of human diseases. The recent discovery of STIM1 and Orai1 as important determinants in store-operated entry is an important breakthrough toward our understanding of the exact molecular identity and mechanism of activation of SOCs. With further appreciation of the molecular makeup and mechanism of activation of store-operated $Ca^{2+}$ influx, we can expect future developments of reagents that specifically target this pathway.

## 3. Activation mechanisms of store-operated entry

At present there are 2 general hypotheses for the activation mechanism of various store-operated entry pathways and of $I_{CRAC}$ in particular: (i) action of a diffusible messenger that is released from the ER to activate plasma membrane channels upon ER $Ca^{2+}$ depletion, and (ii) coupling between proteins within the ER membrane with $Ca^{2+}$ influx channels in the plasma membrane [85,86]. In addition, there is speculation that the activation mechanism may involve insertion of channels into the plasma membrane [153] although some mechanism of initiating the signaling from the ER would still be necessary. There is evidence in support of these ideas in the literature; however, much of this evidence is indirect and discrepancies abound [86]. Direct proof of any of these activation mechanisms as they relate to store-operated entry will likely only come as a result of the identification of the molecular nature of individual SOC channels.

### 3.1. Diffusible messenger

The diffusible messenger mechanism was recently the topic of an extensive review [14] and will thus only be briefly addressed here. The theory of a diffusible factor responsible for activating store-operated entry was first implied by Putney and colleagues [127], and evidence for the existence of such a factor was first demonstrated by Randriamampita and Tsien [103]. In this latter work the authors were successful in isolating an acid-extractable factor from activated Jurkat T-lymphocytes, referred to as $Ca^{2+}$ influx factor (CIF), that was capable of activating $Ca^{2+}$ influx when applied to several different cell types. Importantly, further biochemical purification of CIF led to the resolution of a factor that was only effective at evoking $Ca^{2+}$ influx when microinjected into cells [50,131], and thus acted upon an intracellular target. Another significant boost to the CIF story came when it was shown that a biochemically and functionally similar factor could also be isolated from *Saccharomyces cerevisiae* in which the $Ca^{2+}$-ATPase gene pmr1 is mutated, resulting in depletion of intracellular $Ca^{2+}$ stores [26]; thus, CIF production is apparently not specific to a single cell type or species. This study was also the first to show a positive effect of CIF on the development of $I_{CRAC}$ [26]. The mechanism by which CIF activates $Ca^{2+}$ influx remains unclear, although recent work suggests a role for $Ca^{2+}$-independent phospholipase $A_2$ ($iPLA_2$). It was first shown by pharmacological and molecular approaches that inhibition of $iPLA_2$ activity results in suppression of SOC activity [41,112,120]. Smani et al. [119]

then demonstrated that CIF is able to activate store-operated entry by displacing an inhibitory calmodulin from $iPLA_2$, and that lysophospholipid generated by $iPLA_2$ is responsible for directly activating SOC channels. However, the exact mechanism by which CIF activates SOC and whether it constitutes the physiological activation signal for $I_{CRAC}$ awaits the identification of the molecular entity of CIF.

### 3.2. Conformational coupling

The conformational coupling hypothesis, first proposed by Irvine [48] and further developed by Berridge [8], draws analogy to excitation–contraction coupling in skeletal muscle whereby the dihydropyridine-sensitive $Ca^{2+}$ channel in the plasma membrane directly couples with the type 1 ryanodine receptor in the sarcoplasmic reticulum. In the case of store-operated entry, it was proposed that the $IP_3R$ in the ER membrane directly links with putative $Ca^{2+}$ entry channels in the plasma membrane, and that a conformational change of the $IP_3R$ upon ER $Ca^{2+}$ depletion signals the opening of SOC channels. This hypothesis was revised somewhat by Patterson et al. [88] into what has come to be known as the secretion-like coupling model. This secretion-like coupling model differs from the original conformational coupling model in that the former allows for separation between $IP_3Rs$ and SOCs when stores are full, and in response to store depletion the ER, or specific subdomains of the ER, move close to the plasma membrane and allow the interaction to occur between the $IP_3R$ and SOCs. The most direct evidence for such a mechanism comes from studies demonstrating that stabilization of a cortical ring of actin with agents such as jasplakinolide or calyculin A prevents the activation of store-operated entry [88,111], the idea being that this ring of actin prevents the movement of the ER toward the plasma membrane. However, a subsequent exhaustive study in RBL-1 cells failed to demonstrate any effects of cytoskeletal alterations on the development of $I_{CRAC}$ [2]; a similar lack of dependence of store-operated entry on the cytoskeleton had also been previously demonstrated in NIH-3T3 cells [106]. Thus, cell-type differences appear to be important considerations in the conclusions drawn from experiments that evaluate the effects of the cytoskeleton on store-operated entry. Evidence in favor of conformational coupling between the $IP_3R$ and SOC channels was also gleaned from early studies showing that 2-APB, thought at the time to be a specific $IP_3R$ inhibitor [69], prevents thapsigargin-induced $Ca^{2+}$ entry in HEK293 cells [64]. It was subsequently shown that this drug also inhibits the development of $I_{CRAC}$ in Jurkat and RBL cells [2,15,16,96]. However, these effects of 2-APB on store-operated entry can no longer be taken as proof of involvement of the $IP_3R$, since it was later shown that 2-APB has direct inhibitory effects on CRAC channels independently of antagonism of the $IP_3R$ [2,15,96]. Most significantly, it was shown that 2-APB is equally effective at inhibiting store-operated entry and $I_{CRAC}$ in a DT40 B-cell line in which all 3 isoforms of the $IP_3R$ had been genetically ablated, as compared to its inhibitory effect in wild type DT40 cells [15,65]. And in fact the finding that $Ca^{2+}$ entry and $I_{CRAC}$ develop normally in these $IP_3R$ knockout cells [125] provides the strongest evidence yet to indicate that interaction of $Ca^{2+}$ influx channels with the $IP_3R$ is not required for the activation of $I_{CRAC}$. However, this does not preclude the idea that other proteins within the ER membrane may conformationally couple with CRAC channels and achieve $I_{CRAC}$ activation. Significantly, it has recently been shown that STIM1 may act as such a factor, since it has been shown that STIM1 is able to sense $Ca^{2+}$ store levels within the ER lumen, and undergoes a major relocalization toward the plasma membrane in response to $Ca^{2+}$ store depletion [59,110,123,157], as will be addressed in Section 4. Thus, STIM1 may act as the conformational coupler between the ER and CRAC channels, an idea that has been addressed in a recent review [43], and the conformational coupling literature may now need to be readdressed in the context of STIM1 (discussed below).

### 3.3. Vesicle fusion

The vesicle fusion hypothesis postulates that vesicles containing functional SOCs fuse with the plasma membrane upon store depletion, resulting in plasma membrane insertion of active channels. Evidence in support of this mechanism comes from experimental strategies whereby $I_{CRAC}$ or $Ca^{2+}$ influx are measured under conditions in which exocytosis or vesicle fusion should be impaired. For example, it was shown that pharmacological or molecular interference with SNAP-25, a member of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex, antagonizes store-operated currents in *Xenopus* oocytes [153]. It was also shown in HEK 293 cells that botulinum and tetanus neurotoxins, which disrupt SNAP-25 and vesicle-associated membrane protein 2 (VAMP-2), respectively, significantly reduced store-depletion-activated $Ca^{2+}$ entry [1]. However, another study challenged the interpretation of these findings [114]. An important caveat of all studies examining the roles of vesicle trafficking is that it is difficult to distinguish from such studies a role for induced exocytosis in response to store depletion, as is required by the fusion hypothesis, from disruption of constitutive exocytosis which is necessary to maintain the normal complement of membrane components for SOC function. Furthermore, it will be impossible to directly demonstrate store-driven exocytosis of a CRAC channel until the molecular identity of this channel is known. Interestingly, in the case of TRPC channels, several reports have demonstrated that agonists that activate channel activity also result in exocytotic insertion of these channels [10,22,81,84,118], thus further supporting the idea that exocytosis may play an important role in $Ca^{2+}$ entry mechanisms. However, others have failed to observe such increases in membrane expression of TRPC channels in response to activating maneuvers [115,121]. And, as will be discussed in Section 5, it remains unclear whether TRP channels mediate physiological store-operated entry, and in all of the instances cited above, TRP channels were activated by means other than store depletion.

## 4. STIM1 and Orai1: Molecular components of $I_{CRAC}$

Through the use of RNAi technology, two proteins have recently been identified that are necessary for store-operated entry and $I_{CRAC}$ in particular. The first of these proteins was identified simultaneously by two separate groups using RNAi in *Drosophila* S2 cells [110] and in human HeLa cells [59]. Each group found that when the messages for the proteins Stim in *Drosophila* or STIM1 in mammalian cells were targeted using siRNA, cells were unable to activate $Ca^{2+}$ entry following store depletion using thapsigargin.

STIM1 was identified in limited screens using bioinformatics to identify genes with probable ion channel or signaling functions. However, the second protein, termed Orai or CRACM, bears no resemblance or homology to any other functional protein; thus it is not surprising that its discovery came somewhat later, resulting from RNAi screens of the entire *Drosophila* genome [33,143,156]. In the first publication describing this protein, its identification was facilitated by the finding that this particular gene was also the genetic cause of a rare form of severe combined immunodeficiency (SCID) [32,33]. The identification of these two proteins has revitalized the quest to understand the mechanisms controlling $I_{CRAC}$.

### 4.1. STIM1

Roos et al. performed a large scale RNAi screen in *Drosophila* S2 cells by targeting any protein with a transmembrane domain, including the TRP channel family, as well as any protein that had been previously described as playing a role in store-operated entry [110]. Of 170 genes targeted, only Stim was found to be needed for store-operated entry and activation of $I_{CRAC}$ in S2 cells. In a parallel study Liou et al. targeted a total of 2304 proteins. They identified two mammalian Stim homologues, STIM1 and STIM2, whose knockdown reduced store-operated entry [59]. It should be noted that when Roos et al. applied siRNAs against STIM1 and STIM2 in Jurkat cells, STIM1 was essential for store-operated entry whereas loss of STIM2 had no effect [110]. Perhaps the discrepancy between these two results is due to off target effects of the siRNA used in the Liou study, which was prepared by incubating the mRNA for the respective proteins with the protein Dicer and then treating cells with the processed RNA [59]. It is conceivable that one or more pieces of the dicer-processed STIM2 RNA bore significant homology to the STIM1 message. These reports also showed that while treatment of cells with siRNA against STIM1 abolishes store-operated entry, it has no apparent effect on the intracellular $Ca^{2+}$ store content or the resting cytosolic $Ca^{2+}$ concentration [59,110,123].

STIM1 is a type 1a single-pass transmembrane protein [68,146]. It contains several conserved domains, including a sterile-alpha motif (SAM), a coiled coil region, and an EF-hand domain. Interestingly, neither the SAM nor the EF-hand domains bear full resemblance to their respective consensus domains. The SAM domain contains two N-linked glycosylation sites, making it the first glycosylated SAM domain to be identified [68,146]. EF-hand domains normally occur in pairs; however STIM1 only has one. There is some evidence to suggest that STIM1 can homodimerize or heterodimerize with STIM2, and this could bring together the EF-hands from separate molecules to form an intermolecularly paired EF-hand domain [146]. Due to the presence of the putative EF-hand domain and the unlikely prospect of a channel protein containing only one transmembrane domain, STIM1 is thought to be the $Ca^{2+}$ store-sensing component of the $I_{CRAC}$ pathway as opposed to the CRAC channel itself. In agreement with this prediction, mutations in acidic residues within the $Ca^{2+}$ binding pocket of the EF-hand domain of STIM1, which presumably lower the affinity for $Ca^{2+}$, produce constitutive $Ca^{2+}$ entry that is independent of store depletion [59,157]. Additionally, wild-type STIM1 expressed as a fusion with enhanced yellow fluorescent protein (EYFP-STIM1) co-localized at least partially with a marker for ER but rapidly rearranged into punctate structures following store depletion [59]. EF-hand mutants of EYFP-STIM1 that confer constitutive $Ca^{2+}$ entry also appear constitutively in punctate structures, indicating that $Ca^{2+}$ binding is important for the localization of STIM1 [59]. However, without physical evidence of actual $Ca^{2+}$ binding, it must be considered that the EF-hand domain of STIM1 might not bind $Ca^{2+}$ and could serve another purpose.

There is some debate as to the cellular localization of STIM1 following store depletion. Several groups have reported detection of STIM1 on the cell surface [68,123,146,157]. However, others have failed to detect any STIM1 in the plasma membrane [59,73]. Additionally, while Zhang et al. [157] reported a significant increase in plasma membrane STIM1 following store depletion, Spassova et al. [123] reported no such increase. Both Manji et al. [68] and Williams et al. [146] reported plasma membrane STIM1, but store depletion was not evaluated in these studies because STIM1 was not known to be involved in store-operated entry at the time. Thus, a portion of cellular STIM1 may be inserted into the plasma membrane, but it is unclear if those STIM1 molecules are important for activation of $I_{CRAC}$.

Although it is clear that STIM1 is a necessary component of $I_{CRAC}$ and store-operated entry, the mechanism by which it acts is still a mystery. Three preliminary models have been proposed to explain how STIM1 might work based upon its cellular localization patterns. All three models have in common that STIM1 is located in the ER membrane prior to store depletion. The first model suggests that STIM1 in the ER complexes with STIM1 in the plasma membrane following store depletion [110]. This model of course requires that some STIM1 is found in the plasma membrane, and that this pool of STIM1 is necessary for activation of $I_{CRAC}$. The second model contends that most or all STIM1 is confined to the ER membrane prior to store depletion. After store depletion however, STIM1 (which is presumably no longer bound to $Ca^{2+}$) is somehow transported to and inserted into the plasma membrane [157]. It is not clear what mechanism would be involved in the transport of STIM1 to the plasma membrane, as accumulating evidence suggests that the most likely mechanism, vesicle trafficking, is not involved in $I_{CRAC}$ [2,86]. Both the first and second models are supported by

cell surface biotinylation experiments that detected STIM1 [123,157]. However, these data must be interpreted with caution since STIM1 may have been co-precipitated with a cell surface protein such as the channel itself rather than being directly biotinylated. In studies employing transfection of HEK293 cells with STIM1 and Orai1 (discussed in the following section), no STIM1 was detected in the plasma membrane by antibody binding, despite the generation of huge STIM1- and Orai1-dependent $I_{crac}$-like currents [73]. These latter findings favor a third model which places STIM1 only in the ER membrane [59]. Following store depletion, the punctae seen with EYFP-STIM1 would be very close to the cell surface, but not actually inserted into the plasma membrane. In this scenario the cytosolic C-terminus of STIM1 would interact with additional components of the $I_{CRAC}$ pathway, such as the channels themselves, when brought into the punctae. This model does not exclude the possibility that STIM1 under certain circumstances can be found in the plasma membrane. It does indicate however that this putative plasma membrane pool of STIM1 is not essential for activation of $I_{CRAC}$ and store-operated entry.

### 4.2. Orai1

The second protein component of $I_{CRAC}$ that has been identified was named Orai after the Greek mythological characters the Orai which are the keepers of the gates of heaven [33]. In a report following close thereafter, the name CRACM (for CRAC-Membrane) was suggested [143]. We will use the Orai designation in deference to the first published description of this protein. In addition to Orai1, there are two other Orai family members, Orai2 and Orai3, expressed in mammals [33]. Flies and worms express only one Orai homolog, and this protein most closely resembles the mammalian Orai1 [33]. Orai1 was first identified from genetic linkage analysis performed to identify mutations contributing to a rare form of severe SCID characterized by a lack of $I_{CRAC}$ activity, combined with a genome-wide screen in *Drosophila* to search for genes necessary for nuclear translocation of the $Ca^{2+}$-dependent transcription factor NFAT [32,33]. Orai was identified as essential for $Ca^{2+}$ entry in *Drosophila*, and the role of human Orai1 was confirmed by sequencing the Orai1 gene from the SCID patients as well as 21 of their relatives. In addition to the two SCID patients, 13 of the relatives had lower $I_{CRAC}$ activity in their T cells and were predicted to be heterozygous carriers of the mutation [33]. Importantly, all 13 heterozygous family members as well as the 2 SCID patients carried the same arginine to tryptophan mutation at amino acid position 91 in Orai1 [33]. This mutation was confirmed to block $I_{CRAC}$ by its failure to rescue the lack of $Ca^{2+}$ entry and $I_{CRAC}$ activity when transfected into T cells and fibroblasts from the SCID patients, whereas transfection of the wild-type version did rescue [33].

Orai1, as well as the related Orai2 and Orai3, are predicted to be membrane spanning proteins with 4 transmembrane domains [33,143,156]. Unlike STIM1, Orai1 apparently resides only at the plasma membrane [33,143]. With the exception of the transmembrane segments, these proteins have no other readily identifiable modular domains. Shortly following the report of the role of Orai1 in the human SCID patients, Vig et al. also identified *Drosophila* Orai as an essential component of $I_{CRAC}$, although they called it CRACM1 [143]. In addition to Orai, Vig et al. [143] identified another membrane component that they termed CRACM2; however there is no human ortholog of CRACM2 and so it appears not to be a conserved component of the $I_{CRAC}$ pathway. Subsequently, several reports have demonstrated a remarkable amplification of $I_{CRAC}$ (50–100 fold) when Orai1 is coexpressed with STIM1 [90,122,156]. This amplification is not seen when STIM1 alone is transfected into cells. When Orai1 alone is transfected, there is a clear inhibition of $I_{CRAC}$ in Jurkat [90], RBL [122] cells and HEK293 cells [73] (but see [90]). These findings illustrate several important points. First, they demonstrate that STIM1 and Orai1 are the only components necessary to produce large $I_{CRAC}$ currents when overexpressed, indicating that there are no other rate-limiting components of the system (unless unknown endogenous components are upregulated by the overexpression conditions). Interestingly, the $I_{CRAC}$ currents observed when STIM1 and Orai1 are coexpressed do not completely recapitulate all of the canonical characteristics of $I_{CRAC}$. Most notably, the amplified currents have low permeability for $Ba^{2+}$ and $Sr^{2+}$ [143], both of which are known to pass readily through native $I_{CRAC}$ channels although to a lesser extent than $Ca^{2+}$ [163]. This could indicate that other protein components are found in native $I_{CRAC}$ channels, such as the other Orai family members, that are not necessary for activation and $Ca^{2}$ permeation, but that modulate some of the other pore properties. Second, the finding that Orai1 overexpression alone results in inhibition of $I_{CRAC}$ suggests Orai1 interacts with at least two other similarly limiting components of the SOC pathway, perhaps STIM1 itself. Thus when excess Orai1 is present, the statistical likelihood of combining in an appropriate stoichiometric fashion with these other limiting components is reduced, reducing the number of correctly assembled signaling complexes [101]. Third, the observation that overexpression of these two proteins produces such a dramatic increase in $I_{CRAC}$ current density argues (but does not prove) that Orai1 may be the $I_{CRAC}$ pore-forming unit. If Orai1 were merely another part of the activation machinery then overexpression of STIM1 plus Orai1 might result in a small increase in current density, but only to the point at which the endogenous channels are all in the open state. Especially in HEK293 cells which have nearly undetectable endogenous $I_{CRAC}$, the likelihood of the cells expressing enough endogenous channels to accommodate such large increases in current density seems unlikely.

Mercer et al. [73] reported that Orai2 exhibits properties similar to Orai1; i.e., overexpression of Orai2 alone resulted in inhibition of store-operated $Ca^{2+}$ entry in HEK293 cells, whereas its coexpression with STIM1 resulted in significantly augmented entry and $I_{CRAC}$. The magnitude of the currents with Orai2 were somewhat less than for Orai1, however. Orai3, on the other hand, did not synergistically enhance $Ca^{2+}$ entry when coexpressed with STIM1. However, Orai3 was shown to restore $Ca^{2+}$ entry in cells in which entry was reduced due to RNAi-mediated knockdown of endogenous Orai1, demonstrating that Orai3 is able to function in the $Ca^{2+}$ permeation pathway. Thus,

in transient transfection experiments, the rank order of efficacy of Orai family members appeared to be Orai1 > Orai2 > Orai3 [73].

Our developing appreciation of the critical roles that STIM1 and Orai1 play in the regulation of $I_{CRAC}$ will undoubtedly revolutionize the field of store-operated entry. However, it must be kept in mind that $I_{CRAC}$ may be only one species of store-operated entry that operates physiologically in mammalian cells. Therefore, it is important that we continue to examine other store-operated entry pathways, such as those potentially mediated by TRP channels and other, possibly undiscovered channels. Additionally, it will be interesting to determine whether STIM and Orai isoforms influence store-operated entry pathways that are distinct from $I_{CRAC}$.

## 5. TRP proteins as SOCs

For over a decade, members of the superfamily of TRP proteins, particularly those from the mammalian TRPC family, have been considered molecular candidates of SOCs. Based on amino acid homology, the TRP superfamily includes at present seven families: the TRPC (Canonical; TRPC1–TRPC7), the TRPV (Vanilloid; TRPV1–6), and the TRPM (Melastatin; TRPM1–8) families, and other more distantly related members, like the TRPML (Mucolipin) and TRPP (Polycystin) families, which although not yet well characterized, have received increasing attention due to their association with several inherited human diseases. More recently, the TRPA (Ankyrin) group, with only one mammalian member, and the TRPN (no mechanoreceptor potential C) group, only found in *Caenorhabditis*, *Drosophila* and zebra fish, have been incorporated into the TRP superfamily (reviewed in [89,102]). Recognition of the product of the *Drosophila* trp gene as the $Ca^{2+}$ permeable channel underlying light-induced, PLC-dependent $Ca^{2+}$ entry in fly photoreceptor cells (reviewed in [77]), was rapidly followed by cloning of several mammalian trp counterparts, TRPC1 and TRPC3 being the first to be cloned and expressed in cell lines [13]. Immediately thereafter, realization that ectopically expressed TRPC proteins also make $Ca^{2+}$ permeable cation channels, in most cases activated downstream of PLC stimulation, brought about the belief that these proteins could function as the long-sought SOCs. Consequently, most, if not all studies aimed to elucidate the molecular nature of SOCs focused on mammalian TRPC proteins. However, rather than illuminating, the resultant literature provided controversial evidence for each of the TRPC proteins as well as for TRPV6 and TRPM3, either in favor of or refuting a role as SOCs. To illustrate this controversy, an overview of the major findings regarding the role of TRPs as SOCs is provided below (for a thorough discussion, see [86,102,136,140]).

### 5.1. TRPCs

The TRPC family contains the mammalian genes most closely related to the *Drosophila trp* gene [145,159]. Based on structural similarities the TRPC family is divided into four subgroups: TRPC1; TRPC2; TRPC3, 6 and 7; and TRPC4 and 5. TRPC3, 6 and 7 not only share a high degree of amino acid identity but also functional and regulatory similarities. A structural, and possibly functional relationship also exists between TRPC4 and 5. TRPC2, a pseudo gene in humans and old world primates, forms functional channels in mouse, rat and other mammalian species [161,162]. The reader is referred to a recent review for a comprehensive discussion of structure–function relationships of TRPC channel proteins [140].

The notion that TRPC1 and 4 may form, or be part of SOCs is supported mainly by two different lines of experimental evidence. First, ectopic expression of TRPC1 or 4 in several cell lines increases endogenous store-operated entry induced by either PLC-dependent or pharmacologically-induced depletion of ER $Ca^{2+}$ stores [36,61,91,144,160]. Second, the use of antisense constructs directed against TRPC1 or 4, as well as genetic disruption of the corresponding genes, has efficiently reduced store-operated entry in a variety of cell types [18,19,61,126,132,150,152]. In some instances, those protocols produced a significant reduction of the archetypical store-operated current $I_{CRAC}$ [35,78,93], leading to the conclusion that TRPC1 or 4 are part of native CRAC channels. However, the question remains if the decrease in $I_{CRAC}$ was directly caused by an actual reduction in TRPC1 or 4 protein levels, rather than being the result of compensatory changes in the expression of CRAC channel subunits or regulatory proteins. It is noteworthy that the focused and whole genome screens described above that brought the roles of Stim1 and Orai1 to light [33,59,110,143,156] apparently failed to detect any TRP channels. Moreover, neither TRPC1 nor TRPC4 recapitulate the biophysical properties expected for CRAC channels when ectopically expressed in cell lines. Despite the evidence mentioned above, the role of TRPC4 in store-operated entry has not yet received as much consensus as for TRPC1 (see [3,95] and references therein). This is mainly due to the controversy raised by several studies showing that ectopic expression of mouse TRPC4 in diverse cell lines results in the appearance of PLC-regulated cation channels whose activity is completely independent of $Ca^{2+}$store depletion [80,113,158]. Also, in HEK293 cells, a human epithelial cell line, ectopically expressed human TRPC4 rendered constitutively active channels not regulated by agonist or $Ca^{2+}$ store-depletion [71]. Evidence for store-operated regulation of mouse TRPC5 is limited, and published results are controversial (cf. [92] and [82]). In most instances TRPC5 activation, although downstream of receptor stimulation of PLC, seems not to be related to $Ca^{2+}$ store-depletion [95,113]. A more recent report however, showed that at the single cell level TRPC5 may possibly be activated by a variety of signals, including depletion of $Ca^{2+}$ stores [155]. It is conceivable that the disparate channel behavior, in terms of sensitivity to store depletion, could be influenced by the expression conditions (see below).

Ectopic expression of a splice variant of mouse TRPC2, designated TRPC2A, was shown to enhance store-operated $Ca^{2+}$ entry in monkey kidney fibroblast-like cells [138]. However, a different lab cloned two additional splice variants (named TRPC2α and TRPC2β) [44], and showed that neither these variants nor TRPC2A were able to form channels operated by

$Ca^{2+}$ store-depletion when expressed in HEK293 cells, in conflict with a role for mouse TRPC2 as a SOC. Conversely, native TRPC2 has been reported to take part in endogenous store-operated entry in Chinese hamster ovary cells [37] and spermatic cells [49].

TRPC3 constitutes an inveterate example of the existing controversy as to whether or not a particular TRPC channel can function in a store-operated manner. Some of the confusion regarding the ability of TRPC3 to function in a store-operated mode stems from the fact that, when ectopically expressed, TRPC3 constitutes a channel endowed with significant constitutive activity. This makes it difficult to distinguish a genuine store-dependent effect from mere enhanced constitutive activity, particularly in experiments where $Ca^{2+}$ entry is monitored by means of fluorescent $Ca^{2+}$ indicators (discussed in [100,136]). Despite compelling evidence supporting the view that the signaling to TRPC3 is downstream of receptor-dependent activation of PLC but in a manner not related to $Ca^{2+}$ store depletion (reviewed in [136,140]), studies by Kiselyov et al. [51,52] advocating that TRPC3 channels are indeed store-operated, challenged that view. These studies showed that stable expression of TRPC3 in HEK293 cells results in a non-selective cation channel activated either by stimulation of a Gq-coupled muscarinic receptor or by pharmacological depletion of $Ca^{2+}$ stores, in both instances in an $IP_3R$-dependent fashion. In contrast, Trebak et al. [134] showed that in two different clones of TRPC3-expressing HEK293 cells, including the one used in the studies by Kiselyov et al. [51,52], TRPC3 behaves as a ROC independently of $Ca^{2+}$ store depletion or the $IP_3R$. A possible explanation for these disparate results regarding the store-dependence/independence of TRPC3 channels derives from recent studies by Vazquez et al. [139,142], showing that in the avian B-lymphocyte DT40 cell line ectopic expression of TRPC3 resulted in a channel whose ability to function in either receptor- or store-operated modes was clearly related to channel expression level (discussed in detail in [101]). Store-operation was observed only at very low channel expression levels, whereas conditions that resulted in higher levels of TRPC3 protein expression gave rise to the receptor-regulated mode of TRPC3 activation.

TRPC7 was originally shown to function as a PLC-regulated channel when expressed in HEK293 cells [83], presumably through PLC-derived DAG, but subsequent evidence indicated that it can also function as a SOC [107]. Notably, recent work revealed that these apparently conflicting findings stem from the ability of TRPC7 to operate in either store-operated or receptor-activated modes depending, as for TRPC3, upon the protein expression level [56]. In those studies, Lievremont et al. [56] showed that transient expression of TRPC7 in HEK293 cells rendered a DAG-activated channel, but when expressed stably, it behaved as a SOC. In an attempt to determine which of these two coupling modes corresponds to the physiological function of endogenously expressed TRPC7, a follow up study [57] utilized targeted homologous recombination to knock out TRPC7 in DT40 B-lymphocytes, known to express endogenously both store-operated and non-store-operated pathways [87,125,141]. In what represented the first demonstration of a physiological function for native TRPC7, it was found that knockout of TRPC7 in DT40 B-lymphocytes did not significantly affect $I_{CRAC}$ [57]. Rather, in the avian B cell line, TRPC7 functions as a PLC-regulated, DAG-activated non-selective cation channel. It is possible, however, that native TRPC7 may function as a SOC in other cell types, for instance, when SOC currents exhibit properties reminiscent of TRPCs (see for example [137]).

As of this writing, available evidence indicates that ectopic expression of TRPC6, either of mouse or human origin, results in a non-selective cation channel whose activation is linked to PLC-derived products but is independent of $Ca^{2+}$ store depletion (reviewed in [136,140], but see [22]). It should be noted, however, that the impact of channel expression level on TRPC6 regulation has not yet been addressed.

Evidently, despite some evidence that, at least under certain conditions some TRP proteins may form, or be part of store-operated $Ca^{2+}$-permeable channels, the role of TRPs as genuine SOCs is still under debate. A chronic problem in recognizing TRPs as SOCs is the inability of ectopically expressed TRP proteins to recapitulate $I_{CRAC}$. However, it should be noted that although $I_{CRAC}$ was the first store-operated $Ca^{2+}$ current described and thus stands as the archetypical one, SOCs are heterogeneous with respect to both cation selectivity and pharmacological profile in different cell types (see [86] and refs. therein). This suggests that SOC channels may comprise a heterogeneous group of $Ca^{2+}$-permeable channel proteins. Therefore, it is reasonable to conceive that SOC currents with different properties from $I_{CRAC}$ may indeed involve channels built of TRP proteins. Within this context, it is important to note that functional TRPC channels are thought to be formed by four TRPC proteins that form either homo- or hetero-tetramers (see [140] and references therein) and, regardless of limitations as to which TRPCs can come together to form complete channels, it is recognized that the ultimate tetramer composition can alter functional and pharmacological features of TRPC channels [58,60,124]. Consequently, it is likely that native SOCs result from combinations of endogenous proteins not readily recapitulated in overexpression experiments. Undoubtedly, careful examination of the relationship between TRP protein expression level and channel regulation, as well as more emphasis on determining the composition of native TRP channels by taking advantage of knock out models and/or siRNA strategies, might eventually clarify existing discrepancies that hinder definition of their mode of regulation. However, even in those instances where TRP proteins have been found to be genuinely store-operated, the underlying mechanism coupling store-depletion to channel activation remains unknown. Within that context, in view of the recent discovery that the mammalian $Ca^{2+}$-binding protein STIM1 (see Section 4.1 above) plays an essential role in the activation of SOCs, likely by acting as the sensor of $Ca^{2+}$ store content [59,110], upcoming studies should address the potential role of STIM1 in regulation of store-operated TRPs.

## 6. Role of store-operated entry in $[Ca^{2+}]i$ Oscillations

$[Ca^{2+}]i$ oscillations, which generally consist of a series of repetitive spikes of $[Ca^{2+}]i$, represent the physiological response

of many cells to a wide variety of stimuli [4,6,129,130,148]. Agonist-induced oscillations usually occur as a result of PLC activation followed by the production of $IP_3$ and the repetitive release of $Ca^{2+}$ from internal stores. At high agonist concentrations, it is widely accepted that SOCs are responsible for refilling the ER following store depletion [86,98]. However, the particular role that SOCs play in $[Ca^{2+}]$i oscillations, which occur at submaximal agonist concentrations and involve only a modest depletion of stores, is still a matter of debate.

Since the behavior of $Ca^{2+}$ oscillations varies among different cell types as well as the type of agonist used, it is often difficult to resolve the complex relationship between oscillations and pool depletion. In many cell types, oscillations are abolished when $Ca^{2+}$ is removed from the extracellular medium, suggesting that $Ca^{2+}$ entry across the plasma membrane is necessary to maintain them [12,109,129,149]. It has been proposed that this may be achieved through the action of store-operated entry [5,129]. Conversely, Shuttleworth has provided evidence that agonist-induced oscillations are not mediated by SOCs, but involve $Ca^{2+}$ entry channels regulated by lipid second messengers derived from products of PLC or $PLA_2$ [116]. Others have shown that other non-store operated channels such as TRPs are involved in the maintenance of oscillations [40,108,133,151].

Elimination of $[Ca^{2+}]$i oscillations in $Ca^{2+}$-free external solutions suggests a role for $Ca^{2+}$ entry in maintaining $Ca^{2+}$ oscillations, but does not provide information on the precise nature of the $Ca^{2+}$ entry pathway. In light of this, there is pharmacological evidence to support the involvement of store-operated entry. In one study, hormone-induced oscillations in hepatocytes were inhibited by $Gd^{3+}$, 2-APB, and SK & F 96365, all at concentrations that inhibit SOCs and, specifically, $I_{CRAC}$ [38,39]. In this same study, LOE-908, which blocks non-store operated channels, did not affect oscillations. Others have shown that $La^{3+}$, another inhibitor of SOCs, blocks agonist-induced oscillations in cortical astrocytes [94]. In other studies using similar pharmacological tools, there is evidence that SOCs mediate oscillations in lymphocytes, HEK293 cells, and myotubes [12,28,55]. Whether entry through SOCs initiates or maintains $IP_3$-mediated $Ca^{2+}$ oscillations remains unclear, especially since the molecular identity of the SOCs involved is unknown.

Despite the growing body of evidence to support the role that SOCs play in the maintenance of $[Ca^{2+}]_i$ oscillations, arguments have been raised against their involvement based on two observations. Shuttleworth and colleagues [116,117] maintain that agonist-induced oscillations are predominantly dependent on the generation of AA, a pathway that is activated independently of store depletion. In reports using HEK293 cells that overexpress the M3 muscarinic receptor and pancreatic acinar cells, addition of isotetrandrine, a phospholipase A2 blocker, inhibited the carbachol-induced oscillatory response [74,75]. Moreover, Shuttleworth and colleagues report that with high agonist concentrations, ARC channel activity is lost and the mode of $Ca^{2+}$ entry occurs primarily through SOC activation, a phenomenon termed "reciprocal regulation" [74]. A similar reciprocal relationship between arachidonic acid-activated and store-operated entry was described by Luo et al. [62] and by Moneer et al. [76].

TRP channels, which are associated with both SOC and ROC entry, have also been implicated in the maintenance of oscillations. One study identified a role for DAG-sensitive TRPC3 activation in the initiation of oscillations in glial cells [40]. OAG (1-oleoyl-2-acetyl-*sn*-glycerol; a synthetic DAG analog) -induced oscillations were preserved when external $Ca^{2+}$ was replaced with $Sr^{2+}$, providing further evidence for TRPC channel involvement since these channels are more permeable to $Sr^{2+}$ cations than are SOCs. In another study using antisense technology to silence endogenous TRPC4, carbachol-induced oscillations were damped in HEK-293 cells in a manner similar to the run-down of oscillations in wild type HEK293 cells in $Ca^{2+}$-free conditions [151].

The information encoded by $[Ca^{2+}]$i oscillations has been shown to control a variety of processes including gene transcription, muscle contraction, and differentiation. Hence, it is of great importance to elucidate the mechanisms underlying their initiation and maintenance. The $Ca^{2+}$ entry during $IP_3$-mediated oscillations is difficult to resolve since the release from stores is small. However, in many cell types, oscillations abruptly cease or gradually run down when $Ca^{2+}$ is removed from the external environment, making it clear that entry from the outside is a necessary component in this type of signaling process [42]. With the recent findings that STIM1 and Orai1 play an essential role in $I_{CRAC}$ regulation (see above), these gene products may provide further clues as to the regulation of agonist-induced oscillations.

## 7. Pathophysiology associated with store-operated entry

Channelopathies are commonly the direct cause of disease, or contribute indirectly to the development of a disease. Knowing the extreme importance of SOC and CRAC channel activities in a variety of physiological functions, one would expect to see a pathophysiological phenotype if impaired store-operated entry occurred. The only documented disease state directly associated with a dysfunction in store-operated entry is a hereditary form of SCID syndrome [30,32,33]. SCID refers to a family of pathologies characterized by a malfunction in T and or B-lymphocyte activation and may be caused by a number of molecular abnormalities including mutations in cell surface receptors, signal transduction molecules, and transcription factors [27]. Regardless of the etiology of the condition, SCID patients share in common a hypersensitivity to infection due to compromised immune function. The form of SCID that is related to a dysfunction of store-operated entry was first attributed to a defect in nuclear import of the transcription factor NFAT in T-lymphocytes derived from affected patients [30]. In T-lymphocytes from normal individuals, presentation of an antigen causes a series of synchronized events, including the production of $IP_3$ and release of $Ca^{2+}$ from the ER. This release of $Ca^{2+}$ from the internal stores triggers influx of $Ca^{2+}$ through CRAC channels, and this $Ca^{2+}$ influx activates, via calmodulin, the phosphatase calcineurin which in turn dephosphorylates NFAT and induces nuclear import of the transcription factor

[31,67]. It was subsequently shown that the T-lymphocytes from patients suffering from this hereditary form of SCID completely lack $I_{CRAC}$ [32]. Further, it was recently shown using a combination of two unbiased genome-wide approaches that a missense mutation, whereby a conserved arginine residue is replaced with a tryptophan at position 91 of the Orai1 gene, is responsible for the defect in $I_{CRAC}$ in these patients [33]. Thus, not only has this series of studies with SCID patients led to the discovery of the molecular basis of a rare and debilitating disease, it has also proven instrumental in the discovery of a critical factor, Orai1, involved in the regulation of $I_{CRAC}$.

There is also evidence for a role of store-operated entry in diverse disease states such as acute pancreatitis [104] and Alzheimer's disease [70,72,154], as well as a possible role in causing some of the toxic effects of environmental chemicals that alter $Ca^{2+}$ homeostasis [86]. Furthermore, there are to date four known channelopathies directly associated with TRP channels [79]. Three of these channelopathies involve TRPM6, TRPP2, and TRPML1, which have no known functions in store-operated entry. However, of particular interest to this discussion is the finding that TRPC6 is essential in the regulation of renal podocyte structure and function, and that a mutation in this channel that results in larger TRPC6 currents leads to proteinuria followed by progressive kidney failure [105,147]. However, as stated earlier, there is as yet no evidence to indicate that TRPC6 can function as a SOC under any conditions; therefore, it is not clear whether this defect in TRPC6 reflects a dysfunction in store-operated entry.

## 8. Conclusions

It has been 20 years since the commencement of study of store-operated $Ca^{2+}$ entry pathways. And in that time, an extensive literature addressing properties of various SOC currents, pharmacological and cell biological properties of SOCs, and physiological and pathophysiological roles for store-operated entry has developed. However, until the very recent identification of roles for STIM1 and Orai1 in physiological $I_{CRAC}$, the field has suffered from a lack of unequivocal identification of molecular components of store-operated entry pathways. Thus, these are exciting times for the field of store-operated $Ca^{2+}$ influx. The immediate challenge is of course to precisely define the mechanisms by which STIM1 and Orai1 regulate $I_{CRAC}$, as well as possibly other store-dependent entry pathways (Fig. 1). But beyond that, we are also faced with the task of revisiting much of this literature that has accumulated over the past 20 years in order to determine how the known functions of STIM1 and Orai1 may help address some of the outstanding, unresolved issues. For example, does STIM1 function as a universal $Ca^{2+}$ sensor, capable of activating mechanisms of store-operated entry other than $I_{CRAC}$? And if Orai1 is indeed the CRAC channel, then how do molecular determinants within the protein sequence explain the electrophysiological properties of $I_{CRAC}$ such as permeation and ionic selectivity? Answers to these questions and many more will significantly enhance our understanding of cellular $Ca^{2+}$ signaling and its contribution to normal human health and disease.

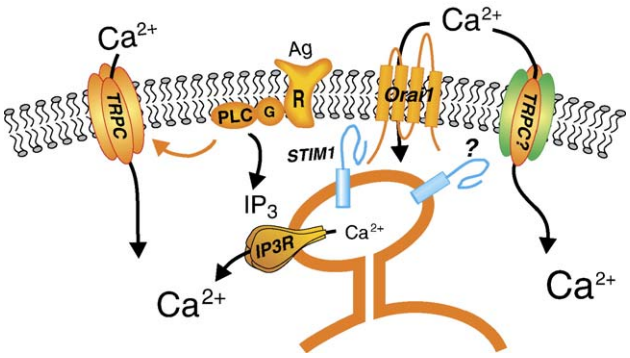


Fig. 1. Proposed model of regulation of store-operated $Ca^{2+}$ entry by STIM1 and Orai1. Agonist (Ag) stimulation of plasma membrane receptors (R) induces PLC activity, the products of which can directly activate $Ca^{2+}$ entry via TRPC channels in a receptor-operated fashion. Further, $IP_3$ generated by PLC induces release of $Ca^{2+}$ from the ER via the $IP_3R$. STIM1, located within the ER membrane, senses ER $Ca^{2+}$ depletion and translocates toward the plasma membrane, where it interacts with and activates Orai1, the putative CRAC channel. It remains to be determined whether STIM1 also regulates other store-dependent entry pathways, such as those mediated by store-operated TRPC channels.

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