TRPC1: a core component of store-operated calcium channels

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Abstract

The TRPC (transient receptor potential canonical) proteins are activated in response to agonist-stimulated PIP$_2$ (phosphatidylinositol 4,5-bisphosphate) hydrolysis and have been suggested as candidate components of the elusive SOC (store-operated calcium channel). TRPC1 is currently the strongest candidate component of SOC. Endogenous TRPC1 has been shown to contribute to SOCE (store-operated calcium entry) in several different cell types. However, the mechanisms involved in the regulation of TRPC1 and its exact physiological function have yet to be established. Studies from our laboratory and several others have demonstrated that TRPC1 is assembled in a signalling complex with key calcium signalling proteins in functionally specific plasma membrane microdomains. Furthermore, critical interactions between TRPC1 monomers as well as interactions between TRPC1 and other proteins determine the surface expression and function of TRPC1-containing channels. Recent studies have revealed novel regulators of TRPC1-containing SOCs and have demonstrated a common molecular basis for the regulation of CRAC (calcium-release-activated calcium) and SOC channels. In the present paper, we will revisit the role of TRPC1 in SOCE and discuss how studies with TRPC1 provide an experimental basis for validating the mechanism of SOCE.

SOCE (store-operated calcium entry): concepts and questions

Cytoplasmic Ca$^{2+}$ concentration [$\text{Ca}^{2+}$$_i$], (intracellular Ca$^{2+}$ concentration), and more specifically regulated increases in [$\text{Ca}^{2+}$$_i$], control a variety of cellular functions [1–3]. Although the ER (endoplasmic reticulum) provides a large intracellular Ca$^{2+}$ store from which Ca$^{2+}$ can be released under a number of different conditions, cells depend on critical Ca$^{2+}$ entry mechanisms for functionally specific and spatially controlled increases in [$\text{Ca}^{2+}$$_i$]. Resting cells generally have low membrane permeability to Ca$^{2+}$, which increases following stimulation of cells by a wide variety of extracellular ligands as well as physical and chemical stimuli due to activation of Ca$^{2+}$-permeable ion channels in the PM (plasma membrane). While several different pathways have been identified for Ca$^{2+}$ influx in neuronal and non-neuronal cells, the present review will focus on SOCE that is stimulated in response to depletion of Ca$^{2+}$ from intracellular Ca$^{2+}$ stores and is mediated via the activation of specific PM channels, termed SOCs (store-operated calcium channels) [2]. SOCE and SOC(s) appear to be ubiquitously expressed in all cell types. However, and somewhat uniquely, the channel characteristics are diverse in different cell types. The first SOCE mechanism to be described, the CRAC (calcium-release-activated calcium) channel, carries a highly Ca$^{2+}$-selective calcium current that is activated in response to internal Ca$^{2+}$ store depletion in T-lymphocytes and RBL (rat basophilic leukaemia) cells [4,5]. Hence, the methodology and criteria for measuring and identifying SOC function have been primarily based on studies with CRAC. Subsequent studies identified channels with different biophysical characteristics in various cell types [2,6,7]. The physiological basis for this diversity is not clear. An important question that arises is whether all the channels that sense internal Ca$^{2+}$ store depletion are regulated by the same mechanism.

Physiologically, store emptiness is most often evoked by an increase in the level of IP$_3$ (inositol trisphosphate) by stimulation of a variety of cell surface receptors. All these receptors are associated with the activation of PLC (phospholipase C) and hydrolysis of the PM lipid PIP$_2$ (phosphatidylinositol 4,5-bisphosphate), which results in the generation of IP$_3$ and DAG (diacylglycerol). IP$_3$ induces release of Ca$^{2+}$ from the ER via activation of IP$_3$R (IP$_3$ receptor) [1–3,8]. However, other mechanisms can also induce internal Ca$^{2+}$ store release and activate Ca$^{2+}$ entry. Recently, protein synthesis has been associated with release of ER Ca$^{2+}$ and activation of SOCE. These studies reveal for the first time a physiologically relevant mechanism that can induce receptor-independent depletion of the internal store. Termination of protein synthesis and release of nascent polypeptide are associated with a change in the ion permeability of the ribosome–translocon complex, resulting in Ca$^{2+}$ release from the ER [9–11]. Thus, in addition to regulating

Key words: calcium-release-activated calcium channel (CRAC channel), calcium signalling, endoplasmic reticulum, regulatory protein complex, store-operated calcium entry (SOCE), transient receptor potential canonical 1 (TRPC1), abbreviations used: [$\text{Ca}^{2+}$$_i$], intracellular Ca$^{2+}$ concentration; CaM, calmodulin; CaM-binding domain, CRAC, calcium-release-activated calcium; ER, endoplasmic reticulum; IP$_3$, inositol trisphosphate; IP$_3$R, IP$_3$ receptor; LRD, lipid raft domain; PIP$_2$, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; PM, plasma membrane; PMCA, PM Ca$^{2+}$ pump; SERCA, sarco/endoplasmic reticulum Ca$^{2+}$-ATPase; siRNA, small interfering RNA; SOC, store-operated calcium channel; SOCE, store-operated calcium entry; STIM1, stromal interacting protein 1; TRPC1, transient receptor potential canonical 1.

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a number of neurotransmitter-dependent physiological processes, e.g. T-lymphocyte activation, smooth-muscle contraction, endothelial-cell permeability, salivary gland fluid secretion etc., SOCE can also sustain optimal levels of protein synthesis by maintaining ER [Ca$^{2+}$]. The latter is a critical factor in the regulation of cell growth and proliferation.

Despite intense focus on SOCE over the past two decades, neither the mechanism by which the status of Ca$^{2+}$ in the ER is transmitted to the PM, to activate or inactivate SOC, nor the molecular components of the channels have yet been identified. Among the many models proposed to describe this elusive pathway, three have received most attention. (i) Conformational coupling hypothesis, which suggests that ER signal is relayed to the SOC via a direct interaction of the channel with IP$_3$R in the ER. (ii) The diffusible factor hypothesis suggests that a factor is released or generated in response to internal Ca$^{2+}$ store depletion. (iii) The secretion coupling model proposes regulated recruitment of channels by fusion of intracellular vesicles [2,3,8,12]. The currently available data do not conclusively establish or exclude any of the three models. A major roadblock towards understanding this fundamental cellular mechanism has been the lack of knowledge of the channel or accessory regulatory components. Over the past decade, several essential components of SOCE have been identified. Members of the TRPC (transient receptor potential canonical) subfamily of cation channels have emerged as central players in this process [2,3,7,13–16]. The rest of this review will highlight significant findings that establish TRPC1 as a core component of SOCE.

Search for SOC: the TRPC1 connection

Studies with Drosophila TRP channel, which is activated by a PLC-dependent mechanism, fuelled the search for mammalian TRP channels, which were suggested as candidate components of the elusive SOC. Consistent with the initial prediction, seven channels (TRPC1–TRPC7) were identified that could be activated in response to stimulation of PIP$_2$ hydrolysis by stimulation of various PM receptors in different tissues [2,3,14,15]. However, only some of them appear to function as SOC. One of the major criteria for identification of SOCs has been activation by ER depletion itself, in the absence of receptor-mediated PIP$_2$ hydrolysis [2]. This is typically achieved experimentally by treatment of cells with thapsigargin, which blocks the ER Ca$^{2+}$ pump and induces Ca$^{2+}$ release from the ER via a leak pathway that might involve the translocon complex [9–11]. Other TRPCs contribute to agonist-stimulated store-operated as well as non-store-operated Ca$^{2+}$ entry mechanisms [2,3,15,16]. It is important to recognize that the function and regulation of TRP channels have not yet been established and there is considerable conflict regarding the mode of activation of a number of these channels. However, the majority of studies with TRPCs involve heterologously expressed channels and relatively less information is available regarding the status of endogenous TRPC channels. Generation of knockout mice models for these channels should greatly help to elucidate their function and also resolve some of the existing controversies.

TRPC1, the first mammalian TRPC protein to be identified, is widely expressed in neuronal as well as non-neuronal tissues including muscle. On the basis of all the studies reported until now for the various TRPC proteins, the results demonstrating endogenous TRPC1 as a SOC component have been most consistent. TRPC1 has been demonstrated to contribute to SOCE in a variety of cell types, including salivary gland, keratinocytes, smooth muscle, DT40, HEK-293 (human embryonic kidney cells), neuronal and endothelial cells, and platelets [7,15,17–24]. In contrast, some studies, primarily with the heterologously expressed protein, describe store-independent regulation of TRPC1 [15,16]. TRPC1 forms diverse SOCs ranging from relatively selective to non-selective (Ca$^{2+}$ versus Na$^+$). Some TRPC1-SOCs display strong anomalous mole-fraction behaviour for Ca$^{2+}$/Na$^+$ permeability which renders them permeable to Ca$^{2+}$ (not Na$^+$) influx under physiological conditions [6]. Furthermore, TRPC1-dependent regulation of K$_{Ca}$ channels, fluid secretion, and smooth-muscle contraction has been demonstrated [7,13]. Thus TRPC1 channels mediate physiologically significant increases in [Ca$^{2+}$]. Recent studies demonstrate novel roles for TRPC1 in cell proliferation, differentiation, and protection against cell death [25–29].

Role of TRPC1 in SOCE

Strategies used for demonstrating involvement of endogenous TRPC1 in SOCE utilize both overexpression and knockdown of the protein. We have shown previously that overexpression of TRPC1 in HSG cells or in vivo in rat submandibular gland cells resulted in a 2-fold increase in the levels of TRPC1 protein and both agonist- and thapsigargin-stimulated Ca$^{2+}$ entry [30]. More importantly, TRPC1 increased the store-operated calcium current $i_{SOCE}$, without altering its characteristics. Similar effects of TRPC1 overexpression were shown with endothelial and smooth-muscle cells [7]. It is important to note that the characteristics of the endogenous SOC in that particular cell are not altered by these manoeuvres. However, overexpression of TRPC1 has not consistently resulted in increased SOCE. In some studies, it appeared that the protein was not in the PM. One reason for lack of effect of TRPC1 overexpression could be that the cells used for these studies might already have significant SOCE, which in many cases involves TRPC1. Secondly, critical components required for SOC formation could be limiting. However, the function of endogenous TRPC1 has been more clearly demonstrated in a large number of studies with cell lines and primary cell cultures by using knockdown strategies [with antisense constructs or siRNA (small interfering RNA)] or by expression of dominant-negative mutants [7,18–20,22,23,26,29]. An additional approach has been to use anti-TRPC1 antibody directed against the pore region of the channel [7]. Thus there is substantial evidence for the requirement of endogenous TRPC1 protein in SOCE, including SOC activity. However, these results did not establish
that TRPC1 is a pore-forming unit of SOC. To clarify this function of TRPC1, we have earlier examined the functional relevance of the proposed pore domain in TRPC1 by either deleting this domain completely or mutating the negatively charged amino acid residues. These mutant TRPC1 proteins when expressed in cells induced a dominant-negative effect on SOCE or changed the Ca\(^{2+}\) permeability respectively [30]. These results conclusively demonstrated that TRPC1 is a component of the SOC in salivary gland cells.

SOCE is subject to inactivation by elevated intracellular Ca\(^{2+}\) concentrations. Consistent with the involvement of TRPC1 in SOCE, TRPC1 is inactivated by Ca\(^{2+}\) via CaM (calmodulin)-dependent feedback inhibition [31]. Furthermore, two binding sites have been identified on the TRPC1 C-terminus: CaMBD1 (CaM-binding domain 1) and CaMBD2. Strikingly, deletion of CaMBD2, but not CaMBD1, attenuated Ca\(^{2+}\)-dependent inhibition of SOCE in human submandibular gland cells, suggesting that it is this second binding site that is most important in the Ca\(^{2+}\)-dependent inactivation of TRPC1 [31]. Additionally, CaMBD1 has also been proposed to be involved in IP\(_3\)-dependent activation of the channel, since the binding of CaM and IP\(_3\)R overlap on TRPC1 [32]. Activation of the channel has been proposed to involve dissociation of CaM and increased association of IP\(_3\)R. It is interesting that other functions for CaM, such as mediation of the delay between Ca\(^{2+}\)-release and -activation of SOCE, has been suggested. Whether this is due to regulated changes in CaM association with TRPC1 or IP\(_3\)R is not yet known.

**Molecular basis for different SOC channels**

TRPC monomers interact via their N-terminal domains to form homomeric and heteromeric channels. Interactions between TRPCs are selective and are most likely to be related to the specific physiological function served by the channel in a particular cell type. The exact molecular signals that dictate these selective interactions between TRP monomers are not yet known. In addition to generating channels with different biophysical properties, such interactions also affect PM localization of the channel. The homomeric TRPC1 channel involves N-terminal coiled-coil domain interactions, whereas the TRPC1–TRPC3 heteromeric interaction involves the first ankyrin repeat region in TRPC1. Although TRPC1 was reported to interact with TRPC4 and TRPC5, and TRPC3 was reported to interact with TRPC6 and TRPC7 [33], complexes of TRPC1 with TRPC3/TRPC6 or TRPC4/TRPC5 have been found in embryonic brain [34]. Not too much information is available regarding the exact amino-acid sequences that are involved in these interactions or the function of these endogenous homomeric and heteromeric TRPCs. There is convincing evidence to show that heteromeric TRPC3–TRPC1 channels are involved in SOCE in HSY cells [23] and hippocampal neuronal cells [28]; TRPC1–TRPC4 channels mediate SOCE in endothelial cells [7]. Thus TRPC1 can interact with other TRPC monomers to generate distinct SOCs. These studies provide a molecular basis by which the previously described diversity in SOC channels can be explained.

**Validation of activation mechanisms**

TRPC1 has also been used to assess the validity of the models proposed for regulation of SOCE. There is considerable evidence for the conformational coupling hypothesis and the secretion model in store-dependent activation of TRPC1, but none supporting the involvement of a diffusible factor. Results published from several laboratories demonstrate that TRPC1 can interact with IP\(_3\)R, consistent with the first model [13,15]. Co-immunoprecipitation as well as direct interactions between the two proteins has been reported. Furthermore, Brownlow and Sage [35] reported that store depletion induced an association between TRPC1 and IP\(_3\)R. In contrast, Yuan et al. [36] suggested that the two proteins were associated in the resting state and that the association was mediated via Homer, which linked TRPC1 to IP\(_3\)R. Further, they provide strong evidence to show that disruption of Homer–TRPC1 interaction leads to activation of SOCE. Thus these data are in support of the conformational coupling hypothesis for activation of TRPC1-SOCE channels. Additionally, data have also been reported that suggest regulated recruitment of TRPC1 in response to cell stimulation. There are two studies that demonstrate trafficking of TRPC1. Rosado et al. [17] report that vesicular trafficking and fusion proteins are associated with TRPC1 in platelets. Further, they show that SNAP-25 (25 kDa synaptosome-associated protein) is involved in activation of TRPC1 and its interaction with IP\(_3\)R. In another study, Mehta et al. [37] report that surface expression of TRPC1, and its association with RhoA and IP\(_3\)R, is increased in response to thrombin stimulation. Rho activates SOCE via regulation of TRPC1 that is required for thrombin-induced increase in endothelial permeability. Thus these latter results show that Rho activation signals interaction of IP\(_3\)R with TRPC1 at the PM of endothelial cells, and triggers Ca\(^{2+}\) entry following store depletion. Together, these results demonstrate that conformational coupling and/or or secretion coupling can regulate TRPC1-SOCE. It is interesting that both PM expression and PM function appear to be regulated via interaction with IP\(_3\)R. Although there appears to be some level of controversy regarding how exactly TRPC1–IP\(_3\)R interaction is affected during activation, these results demonstrate another requirement for the activation of SOCE according to the conformational coupling model which is the close apposition of the ER and PM [2,3,12].

There is convincing evidence to show that SOCE occurs within specific spatially segregated microdomains in the cell [1,13,38,39]. One key observation that suggested this was that Ca\(^{2+}\)-influx-dependent refill of internal Ca\(^{2+}\) stores is accomplished with minimal increase in [Ca\(^{2+}\)], unless the SERCA(sarcoplasmic/endoplasmic-reticulumCa\(^{2+}\)-ATPase) pump activity is blocked. This suggested that ER is closely apposed to the PM, enabling Ca\(^{2+}\) that is entering the cell to be rapidly taken up into the ER via the SERCA pump [40]. This suggestion has also been substantiated by biochemical
and morphological data that reveal a junctional domain that includes the PM and peripheral ER where SOCE is regulated. Proteins from the ER, PM, cytosol and cytoskeletal network contribute to the functional architecture of this domain [13,38,39]. Furthermore, PM lipids, such as PIP2 and PIP3, also have critical roles in Ca2+ signalling. Distinct PM lipid domains, LRDs (lipid raft domains) or caveolar LRD (referring to LRDs that contain the cholesterol-binding protein caveolin-1), which contain high concentrations of cholesterol, sphingolipids, have been suggested to provide a platform for the assembly of Ca2+ signalling complexes [41,42] (discussed further below). There is also a general consensus that Ca2+ signalling proteins are assembled in multiprotein complexes within these junctional microdomains [1,12,13,15,39,40,42,43]. Recent data demonstrate that this complex is dynamically regulated during activation of SOCE, which might also involve rearrangement of the local cytoskeleton and microtubule network. Functional association between SOCE, PMCA (PM Ca2+ pump), SERCA and mitochondria have also been reported [2]. These Ca2+ flux systems contribute to regulation of [Ca2+]i, in the vicinity of the channel and thus determine feedback regulation of SOCE by Ca2+. Thus the current concept regarding regulation of SOCE is that alterations in the junctional ER–PM interactions due to changes in the status of the ER Ca2+ store trigger gating of the PM channel [12,13]. Conversely, refill of the ER, and more specifically peripheral ER, results in inactivation of the channel. This concept predicts involvement of an ER sensor protein that will have to interact within the complex and relay the ‘message’ from the ER to the PM channel. Emerging findings suggest that the Ca2+ signalling microdomains and the molecular components involved in regulation of SOCE are peripherally localized. An important point that needs to be addressed is whether depletion of peripheral Ca2+ stores is sufficient for activation of SOCE. If, as the recent data suggest, junctional complexes between ER and PM channels are the centres for regulation of SOCE, it can be proposed that the status of the local ER stores would be the key factor involved in this regulation.

Consistent with the concept of Ca2+-signalling microdomains described above, mammalian TRPC channels interact with key Ca2+-signalling as well as scaffolding proteins and are assembled into large signalling complexes [13,15,37–39,42,43]. Furthermore, as predicted, both PM and ER proteins are found in the TRPC-signalosomes. TRPC1 interacts with IP3Rs, PLCβ, Gq/11, PMCA and CaM. In addition, TRPC1 co-immunoprecipitates with fibroblast growth factor, metabotropic glutamate and bradykinin receptors [7,15,26]. TRPC1 interacts with two scaffolding proteins. The EVH1 [Ena/VASP (vasodilator-stimulated phosphoprotein) homology 1] domain scaffold protein, Homer, binds to TRPC1 and facilitates its interaction with IP3R. Disruption of Homer–TRPC1 interaction is involved in store-dependent activation of TRPC1 [36]. TRPC1 also interacts with caveolin-1, and disruption of caveolar LRDs decreases thapsigargin-stimulated Ca2+ entry [38,42]. In addition, expression of mutant caveolins, lacking the lipid binding domain, or TRPC1 with mutation in the caveolin-binding domain causes mislocalization of TRPC1 [41].

**New players: old connections**

Despite the data with TRPC1 that I have discussed here, there are considerable discrepancies regarding the role of other TRPCs in the SOCE mechanism. Furthermore, although studies have shown that TRPCs provide the molecular basis for several SOCs, there is still some concern that none of the TRPCs appear to generate ICRA-like currents. Note that TRPC1 and TRPC3 have been shown to have a role in ICRA currents in DT40 cells and T-lymphocytes respectively [20,44]. However, these studies were not followed up with additional data. Thus the search for the SOC channel continues. Recently, by using siRNA strategies as well as genetic linkage approaches, two proteins were identified as components of CRAC channels. The first, STIM1 (stromal interacting protein 1), a single transmembrane domain protein that is found both in the PM and ER, was shown to be involved in activating SOCE, including ICRA. Although overexpression of the protein did not cause any increase, knockdown effectively decreased activation of SOCE. STIM1 has an EF-hand domain at its N-terminal end and it has been proposed that this protein can function as a sensor for ER [Ca2+]i [45,46]. Additionally two more proteins, Orai1 and Orai2, were identified that also attenuated CRAC activity when their expression was decreased. Interestingly, mutations in these proteins were associated with defective ICRA in T-lymphocytes isolated from SCID (severe combined immunodeficiency) patients. Orai1 was first proposed as a regulator of SOCE [47,48]. Subsequently, overexpression of Orai1 and STIM1 was shown to induce large increases in SOCE and ICRA, and mutations in negatively charged amino acid residues in the transmembrane domains to cause changes in the Ca2+ permeability of CRAC channel [49,50]. Thus Orai1 has now been suggested to be a core CRAC channel component. Furthermore, it has been reported that these two proteins are sufficient for generation of CRAC channel [49].

Two recent studies have now demonstrated that TRPC1 interacts with STIM1 [51,52]. Huang et al. [52] report that the mechanisms involved in STIM1-dependent activation of SOCE and CRAC channels are identical with those involved in the binding and activation of STIM1 and TRPC1. Activity of STIM1 requires an ERM (ezrin/radixin/moesin) domain, which mediates the selective binding of STIM1 to TRPC1, TRPC2 and TRPC4, but not to TRPC3, TRPC6 or TRPC7. These observations reveal similar regulation of SOCE, CRAC and TRPC-SOC channels by STIM1. In platelets, which have an endogenous TRPC1-SOCE, store depletion induces an increase in TRPC1–STIM1 association, which is required for activation of SOCE and depends on cytoskeletal changes [51]. Interaction of Orai1 and TRPC1 has not yet been reported at the time of writing, neither has a co-immunoprecipitate of endogenous STIM1 and Orai1 been established. Thus the studies identifying new regulatory proteins for SOCE and those directed towards the function...
of TRPC1-SOC converge on the recently identified protein, STIM1. Future studies should be directed towards resolving the molecular interactions involved in store-dependent regulation of TRPC1-SOC. Furthermore, it will be important to understand how Ca\(^{2+}\) signalling mechanisms and TRPC1 trafficking are co-ordinated to achieve activation and inactivation of TRPC1 in response to depletion and refill of internal Ca\(^{2+}\) stores.

**Concluding remarks**

Much effort has been focused on answering the question of whether TRPC1 is a store-operated channel or not. Most likely, STIM1 is an essential component of TRPC1-SOCs and functions as an ER sensor to regulate activation of this channel. Whether STIM1 regulates both homomeric and heteromeric TRPC1-SOCs needs to be determined. Also, it is unclear whether Orai1 and TRPC1 form separate channels and, if so, do they have distinct functions? Despite all the unanswered questions regarding TRPC1 and SOCE, knowledge that TRPC1 is part of SOCE functions? Despite all the unanswered questions regarding TRPC1 and SOCE, knowledge that TRPC1 is part of SOCE represents a significant advance in our understanding of this physiologically critical Ca\(^{2+}\) entry pathway. Future studies will no doubt reveal further players in the functioning of this channel and elucidate how the signal linked to store depletion is actually relayed to TRPC1 for activation of SOCE.

**References**


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