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Invited review

Store-operated calcium channels and pro-inflammatory signals

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Abstract

In non-excitable cells such as T lymphocytes, hepatocytes, mast cells, endothelia and epithelia, the major pathway for calcium (Ca^{2+}) entry is through store-operated Ca^{2+} channels in the plasma membrane. These channels are activated by the emptying of intracellular Ca^{2+} stores, however, neither the gating mechanism nor the downstream targets of these channels has been clear established. Here, I review some of the proposed gating mechanisms of store-operated Ca^{2+} channels and the functional implications in regulating pro-inflammatory signals.

Introduction

Cells use Ca^{2+} as a key messenger to regulate a broad spectrum of vital processes. Changes in cytoplasmic Ca^{2+} can trigger responses as diverse as exocytosis, muscle contraction, enzyme metabolism, gene transcription and cell proliferation[1]. To increase cytoplasmic Ca^{2+} concentration, Ca^{2+} is either released from intracellular stores or enters the cell by crossing the plasma membrane. In excitable cells, like nerve and muscle, calcium (Ca^{2+}) entry is achieved largely through opening the voltage-gated and ligand-gated Ca^{2+} channels[2,3]. The role of these ion channels is well established and the identity of amino acids in channel properties and gating is well known[2,3]. In non-excitable cells, the main mechanism of Ca^{2+} entry is a process known as "capacitative Ca^{2+} entry" or "store-operated Ca^{2+} entry"[4]. Non-excitable cells do not fire action potentials and voltaged Ca^{2+} channels are absent[4]. Instead, store-operated Ca^{2+} entry is vital for driving most Ca^{2+}-driven messengers. However, despite considerable research, neither the gating mechanism nor the molecular identity of these channels has been resolved. Here, I review some of the recent findings and theories of the store-operated Ca^{2+} entry pathway.

Store-operated Ca^{2+} entry/capacitative Ca^{2+} entry

The model of store-operated Ca^{2+} entry in non-excitable cells was first proposed in 1986[5]. The fundamental idea of this model was that the Ca^{2+} influx pathway could be activated by the amount of calcium in the internal store. As the Ca^{2+} concentration falls, a signal is sent from the stores to open the Ca^{2+} channels in the plasma membrane (Figure 1). The first direct evidence in support of the basic tenet of this idea was identified by discovery of a Ca^{2+} current that was activated by store depletion. The underlying Ca^{2+} channels were called calcium release-activated calcium channels (CRAC) and this calcium selective current was called I_{CRAC}[6].

Store-operated Ca^{2+} channels are the major route of Ca^{2+} influx in non-excitable cells and the best characterized store-operated current is $I_{CRAC.}$ CRAC channels are non-voltage-

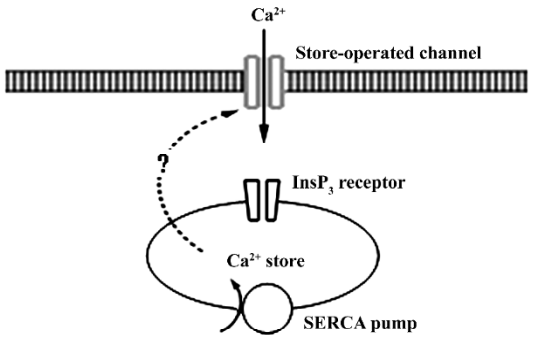

Figure 1. Scheme of capacitative calcium entry/store-operated calcium entry.

gated channels that are very selective to Ca^{2+} ($P_{Ca^{2+}}/P_{Na^+}$> 1000) and have an extremely small conductance for Ca^{2+}[7]. The current is large at negative potentials and approaches the zero current level at positive potentials (>60 mV)[7]. CRAC channels require extracellular Ca^{2+} to maintain their activity. Removal of extracellular Ca^{2+} results in a slow decline of channel activity[8].

Pharmacology of store-operated Ca^{2+} channels Store-operated Ca^{2+} entry can be evoked by any strategy that lowers the Ca^{2+} content of the stores[6,7]. Physiologically, intracellular stores lose Ca^{2+} following a rise in the levels of inositol 1, 4, 5-trisphosphate [Ins(1,4,5)P_3], which opens ligand-gated Ca^{2+} channels in the endoplasmic reticulum (ER) membrane. Experimentally, stores can be easily emptied following application of endoplasmic reticulum Ca^{2+} ATPase (SERCA) inhibitors like thapsigargin or exposure of the cytoplasm to high concentrations of Ca^{2+} chelators such as EGTA or BAPTA that interfere with Ca^{2+} store refilling and cause passive stores to empty. Other methods of emptying intracellular stores include dialyzing the cytosol with Ins(1,4,5)P_3 or permeabilizing the ER membrane to Ca^{2+} by applying Ca^{2+} ionophores like ionomycin.

Establishing a link between ion channel activity and activation of physiological functions relies on the use of pharmacological inhibitors. Thus, the issue of how best to block store-operated Ca^{2+} channels is an important theme in this field. Several pharmacological agents, such as La^{3+}, econazole and SK&F 96365 are known to inhibit store-operated Ca^{2+} channels. However, they can also block several other channels over a similar concentration range[9]. Thus, they should not be considered specific inhibitors for store-operated Ca^{2+} channels. 2-Aminoethoxydiphenyl borate (2-APB), an $InsP_3$ receptor inhibitor, has become a popular tool to investigate store-operated Ca^{2+} channels[10–12]. 2-APB is used as an $InsP_3R$ antagonist, but growing evidence suggests that it is not simply an $InsP_3$ receptors blocker. For example, I_{CRAC} in mutant DT40 cells not expressing $InsP_3$ receptors can be blocked by 2-APB, indicating that targets other than InsP3 receptor are affected[13,14]. Therefore, 2-APB seems to directly block the store-operated Ca^{2+} channels themselves. Another store-operated Ca^{2+} channel inhibitor is diethylstilbestrol. A synthetic estrogen agonist, diethylstilbestrol has been found to inhibit store-operated Ca^{2+} entry in human platelets, rat basophilic leukemia cells, and vascular smooth muscle cells but did not affect a whole-cell monovalent cation current mediated by TRPM7 channels[15]. *Trans*-stilbene, a close structural analog that lacks hydroxyl and ethyl groups, had no effect on store-operated Ca^{2+} influx[15].

Mitochondrial regulation of store-operated Ca^{2+} channels Mitochondria play an important role in production of ATP in eukaryotic cells. However, evidence has revealed that mitochondria can rapidly take up a great amount of calcium that has entered through different Ca^{2+} channels[16–19]. Can store-operated Ca^{2+} channels be regulated by the mitochondria? An elegant series of experiments establishing how mitochondrial Ca^{2+} uptake regulates store-operated Ca^{2+} channels has come from work on rat basophilic leukaemia (RBL) cells and T lymphocytes[20,21]. In RBL cells, mitochondria are also necessary for the activation of I_{CRAC} under physiological conditions of weak intracellular Ca^{2+} buffering. Whole cell dialysis with Ins(1,4,5)P_3 fails to activate any detectable I_{CRAC} unless mitochondria are in an energized state[20,22]. Mitochondrial depolarization using electron transport chain blockers, such as antimycin A or rotenone, prevented I_{CRAC} from developing, and the size of I_{CRAC} to thapsigargin could be increased by energized mitochondria[20,22]. In T lymphocytes, moreover, energized mitochondria reduced Ca^{2+}-dependent slow inactivation, thereby prolonging the time-course of Ca^{2+} influx. Inhibition of mitochondrial Ca^{2+} uptake by CCCP or antimycin A enhance Ca^{2+}-dependent inactivation of I_{CRAC}[21]. Moreover, functional mitochondria are required to sustain CRAC channel activity, and downstream transcription factor NFAT translocation[21].

Mechanism of store-operated Ca^{2+} channel activation

The activation mechanism of store-operated Ca^{2+} channels is one of the most intriguing mysteries. In the past few years, several models have been proposed to explain the link between store emptying and Ca^{2+} influx. Although there is evidence to support each model, there is also evidence that can not be easily fitted into any. In this review, I focus on different activation models (Figure 2) of store-operated Ca^{2+} channels.

Diffusible messenger model Randriamampita and Tsien were the first to propose the existence of a small diffusible molecule that activated store-operated Ca^{2+} channels[23]. This molecule was named calcium influx factor (CIF), and is released following Ca^{2+} store depletion and translocates to the plasma membrane to activate store-operated Ca^{2+} channels. CIF is a phosphorylated molecule that could be degraded by okadaic acid-sensitive protein phosphatases [24]. CIF produced by either mammalian cells or yeast with depleted Ca^{2+} stores directly activates store-operated Ca^{2+} channels in vascular smooth muscle cells[25], and is able to accelerate the development of I_{CRAC} in Jurkat T lymphocytes

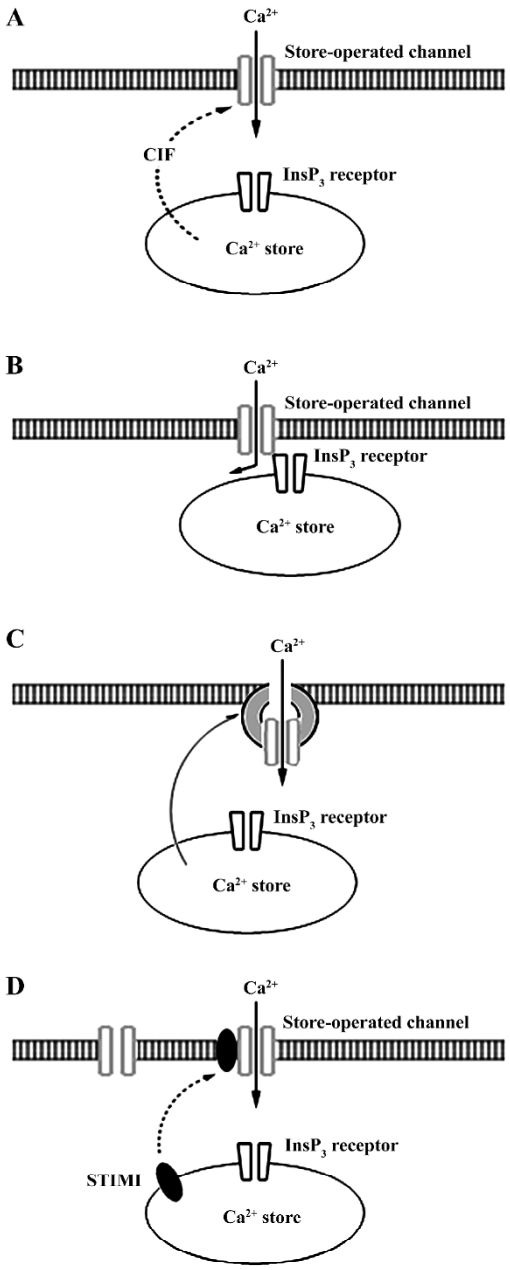

Figure 2. Four models proposed to explain the activation mechanism of store-operated calcium channels. (A) diffusible messenger model; (B) conformational coupling model; (C) vesicular fusion model; (D) calcium sensor model.

and RBL-2H3 cells[26,27]. Although recent work on CIF is encouraging, it is still not known how CIF activates store-operated Ca^{2+} channels. Further evidence in support of the CIF model has come from work in smooth muscle cells[28]. Smani *et al*[28] reported that calcium-independent phospholipase A_2 was essential for CIF-mediated Ca^{2+} influx. In this model, CIF disassociates inhibitory calmodulin from calcium-independent phospolipase A_2 ($iPLA_2$), leading to activation of $iPLA_2$ then LysoPLs, which in turn open store-operated Ca^{2+} channels in the plasma membrane[28]. Importantly, both the pharmacological $iPLA_2$ inhibitor bromoenol lactone and antisense oligonucleotides directed against $iPLA_2$ suppress the activation of store-operated Ca^{2+} channels following either with thapsigargin or CIF[28]. These recent studies support a role of $iPLA_2$ and LysoPLs in the CIF model.

Conformational coupling model Berridge and Irvine[29,30] first proposed a mechanism of connection between ER and store-operated Ca^{2+} channels that involves a direct protein–protein interaction. According to this model, Ins(1,4,5)P_3 receptors in the ER could be physically associated to store-operated Ca^{2+} channels in the plasma membrane. Store emptying could change the conformation of Ins(1,4,5)P_3 receptors, which then regulates the opening of store-operated channels through protein–protein interaction. This idea of conformational coupling was indirectly supported by works on Ins(1,4,5)P_3 receptors and the canonical TRP family, which are candidates for store-operated channels[31,32]. In HEK-293 cells, recombinant TRPC3 channels can be co-immunoprecipitated with Ins(1,4,5)P_3 receptors[33]. Kiselyov *et al*[31,34] also showed that the N-terminal domain of type 1 Ins(1,4,5)P_3 receptors is involved in the activation of TRPC3. Similarly, studies done in human platelets have revealed the interaction between TRPC1 and type II Ins(1,4,5)P3 receptors[32]. However, there are some studies documenting that Ins(1,4,5)P_3 receptors are not essential for the activation of store-operated channels. For example, I_{CRAC} in mutant DT40 cells not expressing all three types of $InsP_3$ receptors can still be activated by thapsigargin, indicating physical coupling might not be essential in the activation of CRAC channels[35]. These findings fit with observations by other groups, all of which reported that heparin, an IP_3 receptor antagonist, did not interfere with the activation of I_{CRAC} in RBL cells or DT40 chicken B cells[36–38]. Thus, in some cell types, conformational coupling between Ins(1,4,5)P_3 receptor and store-operated channels might not be required for the activation of I_{CRAC}.

Vesicular fusion model The third model to explain the activation mechanism of store-operated Ca^{2+} channels is vesicular fusion. This hypothesis suggests that store emptying causes store-operated Ca^{2+} channels to be inserted into the plasma membrane using an exocytotic mechanism. Yao *et al*[39] first reported the vesicular fusion model in *Xenopus* oocytes. They found I_{CRAC} was disrupted by overexpression of a mutant of SNAP-25, indicating functional SNAP-25 is necessary to activate store-operated Ca^{2+} current[39]. In rat megakaryocytes, the vesicular transport in-

hibitor primaquine was found to block the development of I_{CRAC}, suggesting the involvement of exocytotic mechanisms[40]. In HEK-293 cells, Alderton *et al*[41] reported that direct microinjection of botulinum neurotoxin A and tetanus neurotoxin, which cleaves SNAP-25 and specifically hydrolyzes vesicle-associated membrane protein 2, respectively, impaired store-operated Ca^{2+} entry. However, there is evidence indicating that SNAP-25 might not be involved in the activation of store-operated Ca^{2+} entry. The major challenge to the vesicular fusion model came from studies on the expression of SNAP-25 in nonexcitable cells. Scott *et al*[42] found that neither HEK-293 nor COS-1 cell lysates had detectable levels of SNAP-25. In contrast, both HEK-293 and COS-1 cells express high levels of botulinum neurotoxin A-insensitive SNAP-23 protein. In spite of the overexpression of mutant SNAP-23, store-operated Ca^{2+} entry was unaffected. In RBL cells, Bakowski *et al*[43] reported that recombinant protein alpha-SNAP1-285, an inhibitor of exocytosis, inhibited vesicular fusion but had no effect on the activation of $I_{CRAC.}$ Hence, vesicular fusion does not seem to be involved in the activation of I_{CRAC}.

Calcium sensor model In 2005, some exciting results came from Roos *et al*[44] in the elusive mechanism of store-operated Ca^{2+} channels. They applied an RNAi-mediated silencing screen to 170 genes in *Drosophila* S2 cells, including a number of transient receptor potential genes, and used thapsigargin-evoked Ca^{2+} entry as a marker for store-operated channels. One gene coding for a protein called stromal interaction molecule (STIM) significantly reduced thapsigargin-induced store-operated Ca^{2+} influx. They also comfirmed that STIM1 knockout in Jurkat T cells was abolished in $I_{CRAC.}$[44]. Similarly, after screening 2,304 proteins, Liou *et al*[45] identified two proteins, STIM1 and STIM2, that are essential for maintaining the store-operated Ca^{2+} entry in HeLa cells.

The NH_2-terminal domain of STIM1 contains an α helices structure called EF hand motif, and a protein–protein interaction domain called the sterile α motif (SAM)[45]. Because of the EF hand motif, STIM1 is likely to be within the lumen of ER. STIM1 might function as the calcium sensor within stores[45]. By transfecting fluorescent fusion protein to detect the localization of STIM1, Liou *et al*[45] reported the redistribution of YFP-STIM1 into puncta near plasma membrane was triggered by thapsigargin-induced store depletion. Interestingly, when the calcium-binding aspartic acid residue in the EF hand motif was mutated to alanine, store emptying failed to trigger any store-operated Ca^{2+} influx[45].

Zhang *et al*[46] were the first to report that stores depletion results in the translocation of endogenous STIM1 from ER to plasma membrane. They showed that mutation of the EF hand motif that mimics store emptying triggered the activation and translocation of store-operated Ca^{2+} channels[46]. Further evidence supporting the STIM1 model showed that overexpression of STIM1 resulted in a substantial increase in I_{CRAC} and mutants in the EF hand motif and C terminal of STIM1 altered the basic features of CRAC channels[47]. The study indicates that, in addition to being a calcium sensor within ER, STIM1 within the plasma membrane might function as a regulatory component of store-operated Ca^{2+} channels[47]. Thus, a new model proposes that STIM1 is located in the membrane of ER when stores are full. Once stores are empty, Ca^{2+} dissociates from the EF hand motif of STIM1, and STIM1 translocates to plasma membrane to activate CRAC channels. More data supports the involvement of STIM1 in the regulation of store-operated Ca^{2+} channels. Thus, the goal for understanding the mechanism of store-operated Ca^{2+} channels activation seems to be a real possibility.

Physiological function of store-operated Ca^{2+} channels

There is growing evidence for a role of store-operated Ca^{2+} influx in human disease. Severe combined immunodeficiency[48,49]and acute pancreatitis[50,51] have been linked to a failure of store-operated Ca^{2+} entry. Actually, the increase of intracellular Ca^{2+} through CRAC channels can regulate several inflammatory processes. This review will focus on studies over the past several years that have been aimed at understanding the activation of pro-inflammatory signals such as transcription factor nuclear factor κB (NF-κB)[52–54], nuclear factor of activated T cells (NF-AT)[52-54], endothelial nitric oxide synthase (eNOS)[55], and leukotrienes[56] by store-operated Ca^{2+} channels.

NF-κB and NF-AT These two transcription factors play important roles in immunity, cell proliferation and proinflammatory cytokine gene activation[52–54,57–59]. NF-κB is considered to be a pro-inflammatory initiator that can be activated by different stimuli such as lipopolysaccharide[60,61], ER stress[62]. Decoy receptor 3 signalling[63] , tumor necrosis factor[58,59] and variations of the Ca^{2+} oscillations amplitude[52–54]. Similarly, NF-AT is a family of transcription factors that regulate gene expression during the immune response. The activation of NF-AT is tightly regulated by the Ca^{2+}/calmodulin-dependent serine phosphatase calcineurin. In resting cells, phosphorylated NF-AT localizes in the cytoplasm but, after stimulation by calcium rise, calcineurin is activated, resulting in dephosphorylation of NF-AT. NFA-T translocates to the nucleus and stimulates gene transcription[64–66]. Delmetsch *et al*[52] first reported the

involvement of Ca^{2+} oscillations through CRAC channels in the regulation of transcription factors NF-AT, Oct/Oap and NF-κB in T cells. Different frequencies of Ca^{2+} oscillations resulted in different molecular mechanisms for transcription factor activation[52–54]. For example, high frequency of Ca^{2+} oscillations activated all three transcription factors, but low frequency Ca^{2+} oscillations only induced NF-κB activation. The changes in Ca^{2+} oscillations have been extended to control target gene activation, as well as to induce downstream immune response and inflammation. In Jurkat T cells, a sustained Ca^{2+} influx through CRAC channels is critical for transcription of NF-AT and the expression of the interleukin (IL)-2 gene[52–54]. YM-58483, a pyrazole derivative, potently inhibited thapsigargin-induced Ca^{2+} entry, NF-AT transcriptional activity and IL-2 production, but not AP-1-driven promoter activity, indicating that Ca^{2+} entry is able to drive different types of transcription factors[67].

eNOS An increase in vascular permeability is an important sign of acute inflammatory process. Several mediators, such as histamine, bradykinin, prostaglandins and nitric oxide are kown to be involved in vascular permeability changes[68]. eNOS is a calcium/calmodulin-dependent enzyme constitutively expressed in endothelial cells. eNOS-derived nitric oxide has an important role in some of the features of inflammation, such as cell rolling, vascular permeability and angiogenesis[68]. In human endothelial cells, eNOS can be regulated by lysophosphatidylcholine[69–71] through a dynamic interaction between casein kinase 2 and serine/threonine phosphatase 2A in Sp1 binding activity. In pulmonary artery endothelial cells, the SERCA inhibitor thapsigargin can activate nitric oxide production[72]. However, it is not known whether this activation is caused by Ca^{2+} release from intracellular stores or Ca^{2+} influx through store-operated Ca^{2+} channels. The first example of how store-operated Ca^{2+} influx can activate eNOS came from Lin *et al*[73]. Their results indicated that membrane-associated wild-type eNOS enzymatic activity is more sensitive to Ca^{2+} entry through store-operated Ca^{2+} channels than the release from intracellular stores. Because the localization of wild-type nitric oxide synthase is very close to store-operated Ca^{2+} channels, this co-localization could contribute to the rapid activation of nitric oxide synthase by Ca^{2+} entry[73] .

Arachidonic acid and leukotrienes In rat basophilic leukemia cells, Ca^{2+} entry through CRAC channels drivered exocytosis[74]. Among the influential molecules released from mast cells are the leukotrienes that regulate a variety of inflammatory reactions[75]. Chang and Parekh[56] first described the involvement of CRAC channels in the regulation of pro-inflammatory signals by arachidonic acid and leukotriene C_4 in RBL cells. Transient activation of CRAC channels following a 4 min stimulation by thapsigargin was sufficient to generate a significant increase in pro-inflammatory signals. Calcium influx through CRAC channels, but not calcium release from intracellular stores, stimulated arachidonic acid production[56]. Arachidonic acid can be metabolized by 5-lipoxygenase enzyme, leading to the formation of leukotrienes. In the regulation mechanism of LTC_4 secretion, calcium entry through CRAC channels activates extracellular signal regulated kinase (ERKs) within minutes and this is necessary for stimulation of $cPLA_2$ [76]. Ca^{2+} entry activates ERK indirectly, via stimulation of calcium-dependent protein kinase C isozymes α and βI [76]. Following opening of CRAC channels, protein kinase C isozymes α and βI translocate from the cytosol to plasma membrane. Acute inhibition of these isozymes or down regulation following chronic exposure to phorbol ester prevents Ca^{2+} entry from activating ERK, $cPLA_2$ and LTC_4 [76]. Although phorbol ester (PKC activator) stimulation resulted in strong ERK phosphorylation, this was not associated with any arachidonic acid release nor LTC_4 secretion. Therefore, activation of PKC and subsequent phosphorylation of ERK1/2 in the absence of a Ca^{2+} signal is not sufficient to activate $cPLA_2$. Instead, both Ca^{2+} and PKC/ERK are needed. Neither stimulates alone, in the absence of the other, can trigger arachidonic acid and LTC_4 secretion[76]. Importantly, mitochondrial depolarization, by impairing Ca^{2+} entry through CRAC channels, suppressed the phosphorylation of ERK, release of arachidonic acid and downstream LTC_4 secretion. The results establish the importance of mitochondrial regulation of CRAC channel activity in determining subsequent downstream pro-inflammatory molecules[56].

Conclusion

Store-operated Ca^{2+} channels have an essential role in the short-term and long-term regulation of the inflammatory process. In light of the fact that NF-κB, NF-AT, eNOS activation and LTC_4 secretion are very potent pro-inflammatory mediators and have been linked to chronic inflammatory disease such as arthritis, atherosclerosis and asthma, CRAC channels could be a rational therapeutic target for treating such disorders.

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