Store-Operated Calcium Entry: A Tough Nut to CRAC

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Practically every cellular process is directly or indirectly influenced by changes in intracellular free calcium ($[Ca^{2+}]_i$). For the greater part of the past century, the extracellular fluid had been considered the primary Ca^{2+} source, and great emphasis was placed on elucidating just how Ca^{2+} enters cells. However, in the early 1980s, the discovery of the second-messenger function of inositol 1,4,5-trisphosphate (IP_3) revealed the endoplasmic reticulum (ER) as another Ca^{2+} source (*1*). However, it was soon recognized that the release of Ca^{2+} from intracellular stores was often followed by a sustained phase of Ca^{2+} entry from the extracellular space. The intimate link between intracellular Ca^{2+} release and Ca^{2+} influx led to the hypothesis of capacitative or store-operated Ca^{2+} entry (*2*). This hypothesis, that depletion of intracellular Ca^{2+} stores stimulates the subsequent influx of Ca^{2+} across the plasma membrane, received strong support through the identification of a store-operated current, the so-called calcium release–activated calcium current (I_{CRAC}) (*3*). Although anecdotal evidence suggests that other, less selective cation channels may be activated after store depletion, we will limit ourselves to discussing the channels that give rise to the highly Ca^{2+}-selective current I_{CRAC} here, because so far this appears to be the best-characterized store-dependent current (*4*).

Despite hundreds of publications on the topic of store-operated Ca^{2+} entry and intense efforts by many dedicated laboratories, the molecular nature and activation mechanism of store-operated channels (SOCs) remain elusive. In fact, there is such an abundance of hypotheses about candidate activation mechanisms and putative genes that may or may not encode SOCs that the field appears highly confusing and almost impenetrable to outsiders. Even within the field, there is no general consensus about the nature of the store from which the signal emanates, the identity of the putative sensor that monitors the filling state of the stores, the retrograde signal transduction mechanism that activates the SOCs, or the molecular nature of the SOCs. We believe that this is probably due to the enormous complexities of the mechanisms involved in store-operated Ca^{2+} entry, as well as the idiosyncrasies of the experimental methods used to study it.

With this in mind, we will try to briefly summarize what we know about store-operated Ca^{2+} entry (SOCE) and highlight the challenges that the field faces today. We hope that this will lead to a better appreciation of the questions to be addressed by creative minds in the future.

Store-Operated Ca^{2+} Entry

Physiologically, SOCE is initiated either by stimulation of receptors that couple through heterotrimeric GTP-binding proteins (G proteins) to activate phospholipase C–β (PLC-β) or by stimulation of receptors that couple through tyrosine phosphorylation to activate PLC-γ (*4*). This results in phophoinositide breakdown and production of IP_3. This second messenger activates IP_3 receptors, which are ion channels located in intracellular organelles such as the ER. The resulting release of Ca^{2+} into the cytoplasm causes a transient increase in $[Ca^{2+}]_i$, whereas emptying of the stores generates a retrograde signal that activates SOCs in the plasma membrane, which are responsible for the sustained increase in $[Ca^{2+}]_i$ after the initial Ca^{2+} transient (Fig. 1).

Experimentally, one can employ other means to deplete intracellular Ca^{2+} stores; for instance, by blocking smooth ER Ca^{2+} adenosine triphosphatase (SERCA) pumps with thapsigargin or by using Ca^{2+} ionophores (*4*). Store-operated Ca^{2+} entry is often studied in intact cells using Ca^{2+} indicator dyes to measure cytosolic changes in $[Ca^{2+}]_i$. Typically, cells are first exposed to a SERCA inhibitor such as thapsigargin in Ca^{2+}-free extracellular saline, which causes gradual depletion of Ca^{2+} stores through leak pathways. This leads to the activation of SOCs, and when cells are subsequently exposed to saline containing Ca^{2+}, they respond with a large increase in $[Ca^{2+}]_i$ due to Ca^{2+} entry. This may not be the optimal method for assessing SOCs, particularly when used in combination with pharmacological tools of uncertain specificity, because the ensuing change in $[Ca^{2+}]_i$ not only represents a measure of SOC activity but also reflects the net contributions of numerous additional processes that contribute to Ca^{2+} homeostasis.

A more direct way of assessing SOCs is to measure membrane currents electrophysiologically. Typically, this involves whole-cell patch-clamp experiments in which IP_3 is delivered directly into the cytosol through the patch pipette to deplete stores, while extracellular Ca^{2+} concentration is increased to 10 mM and $[Ca^{2+}]_i$ is heavily buffered to increase the amplitude of the exceedingly small CRAC currents and reduce $[Ca^{2+}]_i$-dependent inactivation of CRAC channels. However, extreme care must be taken in the interpretation of even controlled patch-clamp experiments, because it is possible to activate non–store-operated channels under these experimental conditions. The crucial question of whether channels are truly store-operated is difficult to answer unless many different experimental conditions are tested. A case in point that illustrates this problem is the fact that transient receptor potential melastatin 7 (TRPM7) channels have been mistaken for CRAC channels (*5*) because they are activated under the identical experimental conditions previously considered to specifically activate only CRAC channels (*6*). In another example, TRPV6 (CaT1) was considered a candidate CRAC channel because it mediates a current that shares some biophysical characteristics with I_{CRAC} (*7*). However, closer scrutiny of a range of additional biophysical parameters suggests that CaT1 is clearly distinct from CRAC and is not store-operated (*8*).

The enormous complexity at all levels of the signal transduction process remains a challenge to interpretation regardless of the method used to evaluate store-operated Ca^{2+} entry. Moreover, it is possible that different cell types use different mechanisms to regulate the process.

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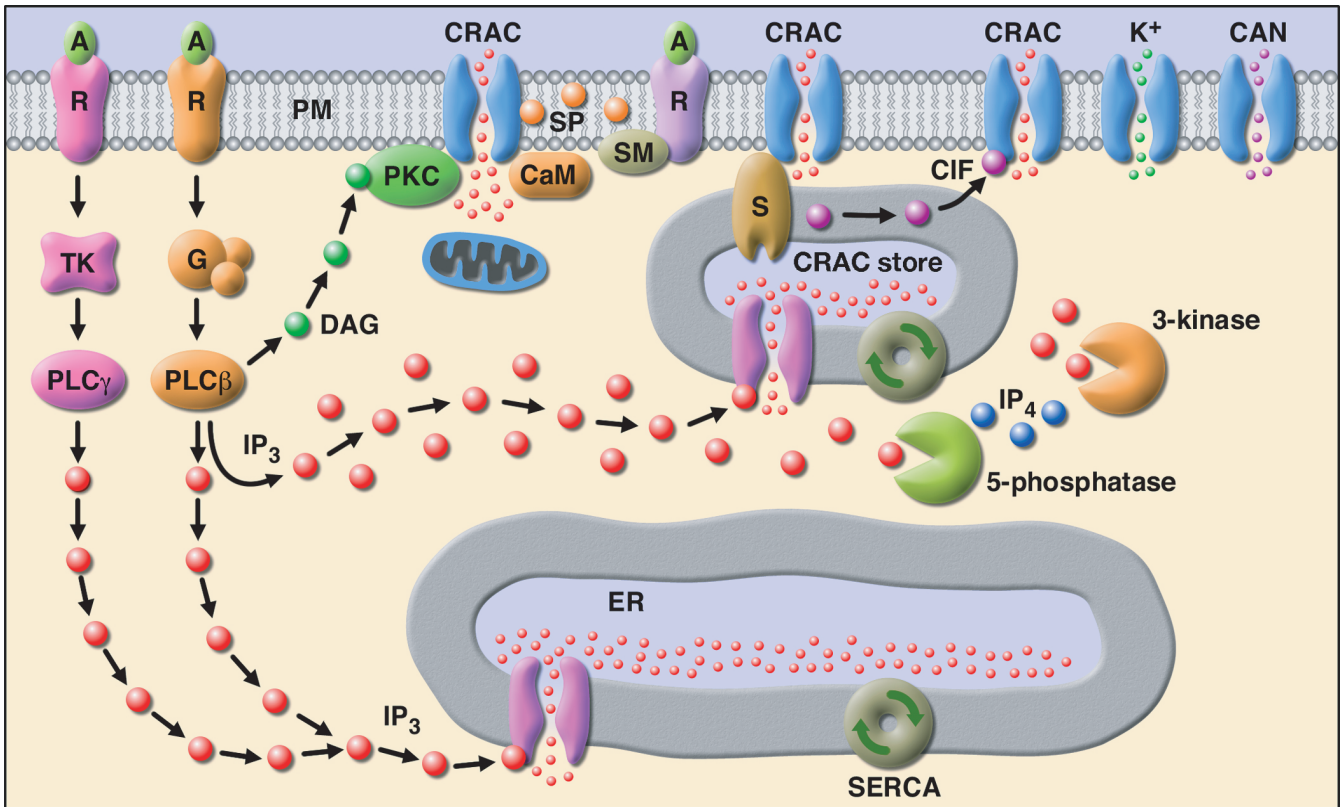

Fig. 1. Store-operated Ca^{2+} entry. Some of the salient features of store-operated Ca^{2+} influx are illustrated, as well as the pathways by which store-operated Ca^{2+} entry is activated and some regulatory mechanisms. PM, plasma membrane; A, agonist; R, receptor; TK, tyrosine kinase; G, G protein; DAG, diacylglycerol; IP_4, inositol 1,3,4,5-tetrakisphosphate; CaM, calmodulin; SM, sphingomyelinase; S, putative Ca^{2+} sensor; CAN, calcium-activated nonselective cation channel; K^+, calcium-activated potassium channel; SP, sphingosine.

IP_3-Sensitive Stores

It is clear that the store from which the retrograde signal for CRAC channel activation emanates must contain IP_3 receptors. However, it is also clear that many cells exhibit considerable heterogeneity among stores and that we may be dealing with more than a single homogeneous store. Patch-clamp experiments in rat basophilic leukemia (RBL) cells have demonstrated that the dose-response relations for IP_3-mediated Ca^{2+} release and I_{CRAC} activation are quite different in that IP_3 concentrations of 1 μM or less empty the bulk of IP_3-sensitive Ca^{2+} stores, whereas I_{CRAC} activation by intracellular IP_3 proceeds essentially in an all-or-none manner, requiring IP_3 concentrations of 3 μM or more (*9*). This suggests that CRAC channels in these cells are under the control of functionally (and possibly physically) distinct "CRAC stores." In the same cells, the sensitivity of these stores to IP_3 may be differentially regulated by local IP_3 metabolism through IP_3 5-phosphatase and phosphoinositide 3-kinase (*10*) and therefore exhibit different response thresholds to IP_3.

The Activation Mechanism of SOC

We are faced with a plethora of hypotheses about possible mechanisms for SOC activation (*4*, *11*). Some evidence has pointed to a fusion mechanism, in which the SOC channels reside in intracellular vesicles and only become integrated into the plasma membrane after the vesicles have fused with the plasma membrane. Other proposals have hinted at the possibility of a direct coupling mechanism of store and plasma membrane proteins, analogous to the model of excitation-contraction coupling in skeletal muscle, where dyhydropyridine receptors and ryanodine receptors interact with each other. Yet another scenario involves the generation of a third messenger, calcium influx factor (CIF). Because evidence for and against each one of these hypotheses has been presented, none of them have gained general acceptance. At this point, it seems that resolution of this issue may have to await the molecular identification of the CRAC channels to enable better testing of what the activation mechanism might be.

CRAC Channels

Once activated, I_{CRAC} is subject to multiple feedback mechanisms, the most immediate of which is the negative feedback exerted by an increase in $[Ca^{2+}]_i$ itself, because CRAC channels undergo direct Ca^{2+}-dependent inactivation (*12*, *13*). Increased $[Ca^{2+}]_i$ has additional, less direct regulatory effects. It can inhibit I_{CRAC} through calmodulin (*14*) or activate other Ca^{2+}-dependent ion channels that can change the membrane potential and thereby affect the driving force for Ca^{2+} entry either positively by hyperpolarization (for instance, by means of Ca^{2+}-activated K^+ channels) or negatively (through Ca^{2+}-activated nonselective cation channels). Mitochondrial Ca^{2+} uptake can also affect I_{CRAC} by acting as a local buffer for $[Ca^{2+}]_i$ (*15*). Further inhibitory effects on CRAC channels are mediated by protein kinase C (PKC)–dependent phosphorylation (*16*) and by sphingomyelinase-mediated production of sphingosine (*17*).

The biophysical properties of I_{CRAC} have been characterized in great detail (*4*). It is a highly Ca^{2+}-selective current with properties that suggest it is due to the activity of ion channels: (i) It conducts Ca^{2+} and to a lesser extent Ba^{2+} and Sr^{2+} and even has some small Mn^{2+} permeability; (ii) it loses selectivity in divalent cation-free extracellular solution, giving rise to large Na^{+} currents; and (iii) ion current changes instantaneously when the membrane potential is changed, suggesting that ions flow through an open pore. The molecular identification of the CRAC channels may hold the key to unravel the entire signaling mechanism underlying store-operated Ca^{2+} entry and naturally is the subject of intense investigation. The transient receptor potential (TRP) family of ion channels was initially considered likely to harbor the elusive CRAC channels, and indeed many of its members have been reported to be store-operated. However, from the available information about TRP channels, no firm candidate has emerged that would fulfill all of the biophysical requirements needed to match the characteristics of I_{CRAC}. Not all TRP channels have been functionally expressed or fully characterized. Thus, it would be premature to rule out the possibility that the TRP family contains the sought-after CRAC channels; however, we should certainly consider alternative avenues that may even go beyond the traditional ion channel concept into the area of ion transporters. In this context, it is noteworthy that I_{CRAC} cannot be resolved at the single-channel level, because the single-channel conductance is well below 1 pS (*12*, *18*). At this level, the boundaries between ion channels and transporters become rather fluid (*19*, *20*), and it is conceivable that CRAC may not necessarily be a classical ion channel but a member of one of the vast number of transporter families. The search is still on, and we should think outside the box to CRAC this very tough nut.

Editor's Note: This Perspective is part of a series related to an E-Conference held at *Science's* STKE. Invited participants were asked to provide Perspectives that summarized the ideas, conclusions, areas of controversy, and challenges for future work that emerged in the online discussion at http://stke.sciencemag.org/cgi/forum-display/stkeforum;14.

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