Exploring the unique features of the ARC channel, a Store-independent Orai channel

Jill L Thompson and Trevor J Shuttleworth*

Department of Pharmacology and Physiology; University of Rochester Medical Center; Rochester, NY USA

Keywords: stromal interacting molecule, Orai1, Orai3, calcium channel, calcium entry, arachidonic acid

Abbreviations: ARC channel, arachidonate-regulated Ca\(^{2+}\) channel; CRAC channel, Ca\(^{2+}\) release-activated Ca\(^{2+}\) channel; STIM1, stromal interacting molecule 1

The discovery of the Orai proteins, and the identification of STIM1 as the molecule that regulates them, was based on their role in the agonist-activated store-operated entry of calcium via the CRAC channels. However, these same proteins are also essential components of the ARC channels responsible for a similar agonist-activated, but store-independent, arachidonic acid-regulated entry of calcium. The fact that these 2 biophysically similar calcium entry pathways frequently co-exist in the same cells suggests that they must each possess different features that allow them to function in distinct ways to regulate specific cellular activities. This review begins to address this question by describing recent findings characterizing the unique features of the ARC channels—their molecular composition, STIM1-dependent activation, and physiological activities—and the importance of defining such features for the accurate therapeutic targeting of these 2 Orai channel subtypes.

The agonist-activated entry of calcium into cells via the highly calcium-selective channels formed by members of the Orai family of proteins represents a critical and highly versatile component of signaling responses in cells, essential for controlling a wide variety of cellular processes including secretion, proliferation, motility, growth, and cell death. To date, 2 such Orai channels have been identified as endogenously present in a variety of different cell types—the extensively studied store-operated CRAC channels, and the less well-known store-independent, arachidonic acid-regulated ARC channels. Although the calcium entry pathway mediating by the ARC channels was first identified in 1996,\(^1\) and the channels biophysically characterized in 2000,\(^2\) our understanding of their activation, regulation, and functional roles is significantly less well-developed compared with that of the CRAC channels. However, it is now becoming increasing clear that delineating the detailed differences between the activation and activities of these 2 channels is critical to providing a detailed understanding of their specific roles in the control of cellular calcium signaling events in both health and disease.

The initial breakthrough in our understanding, at the molecular level, of the activation of store-operated calcium entry mediated via the CRAC channels came with the identification of “stromal interacting molecule 1” (STIM1) as the sensor of calcium store depletion.\(^3-5\) It was, therefore, somewhat unexpected that subsequent data showed that this same molecule was also essential for activation of the calcium store-independent, arachidonic acid-regulated ARC channels\(^6\) (Fig. 1). Less surprising, given the close similarity in their biophysical properties (high calcium selectivity, small conductance, etc.), was the subsequent finding that both channels are formed by members of the Orai family of proteins.\(^7-11\) However, studies involving examination of both store-operated and arachidonate acid-activated currents in cells expressing a variety of different concatenated Orai1/Orai3 constructs revealed that, while the functional CRAC channel is formed by a homotetrameric assembly of Orai1 subunits,\(^12-17\) the ARC channel is a heteropentameric structure comprising 3 Orai1 subunits and 2 Orai3 subunits\(^18\) (Fig. 2). Confirmation that these specific concatenated pentamers were not excluding any subunits on expression in cells was demonstrated by the fact that incorporation of either a single dominant negative Orai1 subunit (E106Q), or the equivalent Orai3 subunit (E81Q) resulted in the complete loss of arachidonic acid-activated currents. Moreover, co-expression of an Orai1-Orai3 dimer with a trimeric construct comprising 2 Orai1 subunits and a single Orai3 resulted in currents indistinguishable from those seen with the above Orai1/Orai3 pentamers. Finally, the currents resulting from the expression of these concatenated pentamers displayed all the key biophysical and pharmacological properties seen with the endogenous ARC channels, including their specific dependence on plasma membrane STIM1.\(^19\)

An interesting question that arises from these findings is why 2 such biophysically similar channels would evolve to apparently co-exist within the same cells. The most obvious answer would seem to be that, although their basic biophysical properties are almost identical, their modes of activation are markedly different—raising the possibility that such differences allow these 2 channels to function in distinct ways to induce and/or modulate specific cellular activities. In this context, the most obvious difference in the activation of the ARC channels compared with the...
CRAC channels is, of course, the absolute dependence of the former on low concentrations of arachidonic acid, coupled with the absence of any effect of calcium store depletion. However, exactly how arachidonic acid acts to induce the activation of the ARC channels is still far from clear. Experiments using a membrane impermeable analog have shown that the arachidonic acid is acting via the intracellular environment, but whether this is a direct effect of the fatty acid, or via some intermediate moiety (an arachidonic acid “receptor” or binding protein) is unknown. Similarly, it is unclear if the action of the arachidonic acid, whether direct or not, is on the Orai proteins that form the channel, or on the STIM1 that is essential for its activation.

Despite these obvious gaps in our understanding of how the channels are activated, recent studies have revealed some differences in the key regions of the channel that are required for such activation. First, experiments involving the expression of various concatenated constructs showed that specificity for activation by arachidonic acid required the presence of 2 Orai3 subunits within the Orai1/Orai3 pentamer. In addition, activation of the CRAC channel is known to involve an action of STIM1 on the N-terminal region of Orai1, specifically the sequence comprised of residues immediately adjacent to the first transmembrane domain Leu74-Ser90 (Li et al. 2007; Park et al. 2009). The corresponding sequence in Orai3 (residues 49–65) is virtually identical, with just a single lysine to an arginine change at the fifth position in the sequence. Given this, the clear expectation was that this sequence would also be the determining region for the STIM1 dependent activation of the ARC channels. However, a series of deletion and chimeric substitution experiments showed that, in marked contrast to the CRAC channels, the arachidonic acid-dependent activation of the ARC channels required the presence of the entire Orai3 N-terminus (Fig. 3). The essential nature of the Orai3 N-termini for selective activation by arachidonic acid was further demonstrated by the fact that, while a concatenated pentameric construct containing just a single Orai3 subunit (a 3-1-1-1-1 construct) was selectively activated by store-depletion, substituting just the N-terminal sequence of one of the Orai1 subunits in this construct with the corresponding sequence of Orai3, was sufficient to result in a channel whose activation is strictly dependent on arachidonic acid and independent of store-depletion.

As noted above, STIM1 is an essential component of the activation of both CRAC and ARC channels. However, consistent with the requirement of calcium store-depletion for their activation, it is now clear that CRAC channel activity depends on the major pool of cellular STIM1 that is located in the membrane of the endoplasmic reticulum (ER). In contrast, activation of the ARC channels is dependent on the pool of STIM1 that constitutively resides in the plasma membrane, and this typically comprises only some 15–25% of total cellular STIM1.

Interestingly, the demonstration of the existence of this plasma membrane pool of STIM1 actually preceded its identification as a key regulator of CRAC channel activity by sensing the depletion of ER calcium stores. In these earlier studies it had been shown that successful delivery and insertion of STIM1 into the plasma membrane depended on the N-linked glycosylation of 2 sites within the N-terminal portion of the molecule (N131 and N171). Correspondingly, using cells in which endogenous STIM1 had been depleted by an appropriate siRNA, the expression of an siRNA-resistant STIM1 construct in which these 2 sites had been mutated to glutamines reduced ARC channel currents to negligible values without affecting the co-existing CRAC channel currents. More recently, evidence has indicated that these 2 pools of STIM1 are not only spatially distinct, but also entirely functionally distinct. Thus, it is clear from simple store-depletion protocols that activation of the ER-resident STIM1 has no effect on the activity of the ARC channels. With respect to CRAC channel activation, it is known that the specific initial step in the normal activation of these channels is the loss of calcium from a calcium-binding EF-hand domain located within the N-terminal portion of STIM1 that lies within the lumen of the ER. Given this, simply exposing cells to a divalent-free extracellular medium would be expected to result in a similar loss of calcium from the EF-hand of STIM1 in the plasma membrane and, therefore, might be expected to result in the activation of CRAC channels. Of course, the absence of extracellular calcium would mean that the normal calcium-selective currents would be absent, but the use of a divalent-free external solution should result in the appearance of the much larger sodium-dependent currents through any active CRAC channels. However, no such currents could be detected under these conditions, a finding that was consistent with data in an earlier study by Bakowski and Parekh. Consequently, it seems that the pool of STIM1 that...
resides in the plasma membrane is apparently incapable of activating the CRAC channels, suggesting that the 2 pools of cellular STIM1 are functionally entirely distinct.

Subsequent investigation of the details of the unique mode of activation of the ARC channels, and the potential implications this might have for their functional activities, has been greatly facilitated by the development of a novel STIM1 construct that exclusively enables the activation of the ARC channels, even under conditions of agonist stimulation that would normally be capable of activating the CRAC channels. For this, we considered that the plasma membrane location of the STIM1 that activates the ARC channels meant that the N-terminal portion of the molecule would lie in the extracellular medium. Here, the relatively stable extracellular calcium concentration of some 1–2 mM would mean that the EF-hand domain of STIM1, with its $K_d$ for calcium of ~300–600 µM, would never normally lose its bound calcium. Consequently, it would seem that this part of the STIM1 molecule that plays such a key role in the initiation of CRAC channel activation, would be unlikely to play any role in the activation of the ARC channels. We, therefore, designed a construct that comprises just the cytosolic portion of STIM1 with a short N-terminally attached sequence based on the SH4 domain of Lck-kinase that enables it to be anchored to the inner face of the plasma membrane (Fig. 4). Expression of this construct, named Lck-STIM1-C, in cells in which the endogenous STIM1 has been depleted by an appropriate siRNA resulted in arachidonic acid (AA)-activated currents that displayed all the features of the native ARC channels, including current magnitude, current-voltage relationship, rate of activation, and sensitivity to arachidonic acid. In contrast, maximal depletion of ER calcium stores (e.g., using adenophostin) or addition of an appropriate agonist (e.g., carbachol) failed to induce any significant CRAC channel activity. Interestingly, further studies showed that the ability of this construct to activate the ARC channels was dependent not only on the tethering to the plasma membrane mediated by the Lck-domain, but also on the specific orientation of the tethered construct with respect to the membrane.

We have now made extensive use of this Lck-STIM1-C construct to examine the molecular basis for the activation of the ARC channels by plasma membrane STIM1, and how this is impacted by the unique location of these 2 components in the same membrane. Initial examination of the specific domains or regions of the cytosolic portions of STIM1 critical for interactions with the Orai proteins that form the channels indicated many similarities between the ARC channels and CRAC channels. Thus, ARC channel activity was essentially eliminated by C-terminal deletions of Lck-STIM1-C up to residue 341 that removed the so-called “calcium activation domain” (CAD domain, ref. 27) formed by the second and third of 3 predicted
Predicted to induce various degrees of disruption of the relevant coiled-coil regions can inhibit interactions with the C-termini of specific Orai proteins resulting in the loss of channel activity. Thus, a single leucine to serine mutation of residue 373 (L373S) in STIM1 results in a markedly reduced ability to interact with the C-terminus of Orai1, and a consequent loss of CRAC channel activity. However, incorporation of the same mutation in the Lck-STIM1-C construct failed to affect ARC channel activity. In contrast, inhibition of the apparently significantly stronger interactions with the C-terminus of Orai3 requires a double mutation (L373S/A376S) in STIM1, and incorporation of this double mutation into Lck-STIM1-C resulted in the loss of ARC channel activity. Together, these data would seem to indicate that, while interactions between the CAD domain of STIM1 and the C-terminal regions of Orai1 are not essential for ARC channel activation, STIM1 interactions with the corresponding regions of Orai3 are essential for activation of the channels. However, this should not be interpreted to indicate that STIM1 in the plasma membrane does not interact with the Orai1 subunits in the ARC channel. Rather, that the absence of such interactions is, in itself, insufficient to inhibit the ability of STIM1 to activate the channel.

Several studies have proposed that, under resting conditions, STIM1 exists as a dimer, a conformation supported by crystallographic studies of the structure of the CAD/SOAR domain which further indicate that such dimers assume a V-shaped conformation (Fig. 5A). Mutation of key residues located within the proposed dimer interface (W430A, I433A, and L436A) which prevent dimerization, result in an inhibition of store-operated calcium entry. Consistent with this proposed constitutive dimerization of STIM1, incorporation of these same mutations in the Lck-STIM1-C construct was similarly inhibitory for AA-activated ARC channel function. Interestingly, the “cleft” formed by this V-shaped conformation of the STIM1 dimer is lined by a series of basic residues located within the second coiled-coil region (residues 382–387)—residues that have previously been shown to be critical in establishing the interactions between STIM1 and the Orai1 C-terminus necessary for CRAC channel activation. Experiments essentially following the approach described by Korzeniowski et al., but now using the ARC channel-specific Lck-STIM1-C construct, demonstrated that mutation of the series of 4 lysine residues that lie within this putative “activation cleft” (K382, and K384–386) to alanines and glycines resulted in a similar profound inhibition of ARC channel currents.

### Figure 3.

The sensitivity of the ARC channel to activation by arachidonic acid depends on the presence of 2 Orai3 N-termini. (A) Diagrams representing the different chimeric Orai subunits used to examine the critical regions of the protein for determining sensitivity of the channel to activation by arachidonic acid. The numbers below represent the composition of these chimeras where the first number (1 or 3) represents the cytosolic N-terminus, the second number represents the transmembrane domains along with their extracellular and intracellular linking “loop” regions, and the third number the C-terminal cytosolic domain. The “(1/3)” and “(3/1)” notations indicate chimeras of just the N-terminal region, as appropriate (see text for details). (B) The mean (± SE) magnitude of AA-activated ARC channel currents measured at -40 mV in cells expressing the concatenated 31113 ARC channel pentamer (black), and in a series of experiments (red) where a 3111 heterotetramer was co-expressed with the relevant monomer as indicated. Expression of a chimeric Orai1 monomer containing just the N-terminus of Orai3 results in currents indistinguishable from those seen on similar co-expression of a full-length Orai3 monomer. However, deletion of the extreme N-terminal portion (X33), or substitution of just part of the N-terminus with the corresponding Orai1 sequence ([1/3]11, or [3/1]11) results in significantly diminished AA-activated currents. Data redrawn from reference 20.
that disruption of this region either by deletion of the sequence comprised of residues 310 to 337, or the mutation of the 4 C-terminal glutamic acids (E318, 319, 320, and 322) to alanines, induced the constitutive activation of calcium entry via CRAC channels, even in the absence of any store-depletion.\textsuperscript{34,36,37} The suggestion was that, under normal circumstances the depletion of ER calcium stores initiated some process, possibly as a result of the oligomerization of STIM1, which induced a conformational change in this autoinhibitory domain, disrupting its activity and allowing STIM1 to interact with and activate the CRAC channels. In marked contrast to the constitutive activation of the CRAC channels following incorporation of the E-to-A mutations described above, expression of a Lck-STIM1-C construct bearing these same mutations failed to induce any constitutive activation of the ARC channels. More surprisingly, expression of this mutant construct even prevented the ability of exogenous arachidonic acid to activate the ARC channels.\textsuperscript{24} Such a pronounced difference in the effect of these mutations in the proposed “autoinhibitory domain” of STIM1 on the activity of CRAC vs. ARC channels suggests that the actual mechanisms of activation of these 2 channel types by STIM1 are fundamentally distinct. As yet, we can only speculate as to what these differences might be. However, one possibility is that they arise from the specific physical orientation of the ARC channels and STIM1 within the same membrane. For example, interesting studies by Muik et al.\textsuperscript{37} report that expression of an intramolecular FRET sensor based on the coiled-coil region of STIM1 assumed a condensed conformation, exhibiting high levels of FRET. However, incorporation of the above acidic residue mutations of the “autoinhibitory domain” within this construct, resulted in a markedly reduced FRET signal, indicative of a change into an extended conformation (Fig. 5A). Such a shift into an extended mode could be critical for the successful presentation of the active site of STIM1 molecules located in the ER-membrane to the appropriate C-terminal regions of Orai1 in the adjacent plasma membrane (Fig. 5B), thereby spanning the gap of ~10–20 nm between these membranes.\textsuperscript{38-41} Indeed, a recent comprehensive structural study has essentially confirmed this basic mechanism of the extension of the STIM1 cytosolic domains following the loss of bound calcium from the luminal EF-hands.\textsuperscript{42} In contrast, assuming such an extended conformation by STIM1 molecules in the plasma membrane might be expected to essentially move the active regions of STIM1 away from the relevant regions of the Orai1 subunits that form the ARC channels located in the same membrane (Fig. 5B). Of course, such proposals are entirely speculative at this stage, and other explanations for these findings are certainly possible. However, they do raise the general point that, because the relevant STIM1 molecules and the ARC channels they activate lie within the same membrane, the nature of the physical interactions between these 2 molecules might differ fundamentally from those for the CRAC channels where the 2 relevant “components” are situated in opposing membranes.

Further evidence for marked differences in the spatial aspects of STIM1-dependent activation of the ARC channels vs. the CRAC channels was revealed by studies examining the physical interactions between STIM1 in the plasma membrane and the channels. Again, these revealed marked differences from the situation demonstrated for activation of the CRAC channels. Clearly, the activation of the CRAC channels by STIM1 in the ER membrane involves a purely transient interaction between these 2 entities that is initiated following ER store depletion, and terminated when the stores replenish. In contrast, studies involving a variety of different approaches (co-immunoprecipitation of the endogenous proteins, co-localization of expressed fluorescently-tagged constructs, bimolecular fluorescence complementation assays, and FRET assays) demonstrated that the plasma membrane STIM1 appears to exist in a constitutive association with the ARC channels, or at least with the Orai3 subunits within the channel\textsuperscript{38} (Fig. 6). Moreover, additional studies revealed that
**Figure 5.** Diagrams illustrating conformational changes in the cytosolic regions of STIM1 that are suggested to occur on activation, and their consequences for CRAC and ARC channel activities. (A) Loss of calcium from the EF-hand, as a result of calcium depletion of the ER store, induces the cytosolic portion of STIM1 to assume an extended conformation. + symbols indicate the so-called “activation cleft.” Mutations of four glutamic acids to alanines within the “autoinhibitory domain” of the first coiled-coil region (green) also induce this conformational change in the absence of loss of calcium from the EF-hand. (B) Such a proposed extended conformation might be important for ER STIM1 to interact with the CRAC channels in their opposing membranes, but would be expected to position the “activation cleft” of plasma membrane STIM1 away from the ARC channels in the same membrane.

The extent of this association was unchanged following maximal activation of the channels either by exogenous application of arachidonic acid, or carbachol-induced receptor activation in cells stably expressing the m3 muscarinic receptor. Once again, these findings suggest fundamental differences in the nature of the activation of the ARC channels compared with that of the CRAC channels. For example, the constitutive association of the channels with STIM1 would seem to suggest that the critical action of arachidonic acid is most likely to induce, or enable, the actual gating of the channel. This clearly differs fundamentally from the situation with the CRAC channels where the essential role of the depletion of intracellular calcium stores is to induce the association of the STIM1 with the channel.

Importantly, these fundamental differences in the overall mode of channel activation might be expected to have additional potential consequences. For example, the demonstrated constitutive association of STIM1 and the Orai3 subunits within the ARC channel (Fig. 6), might be expected to result in a markedly more rapid activation of these channels compared with that of the CRAC channels, which involves the physical translocation of STIM1 molecules within the ER membrane following calcium-store depletion to sites close to the plasma membrane where interactions with, and activation of, the CRAC channels can be initiated. In addition, as already noted, the key pool of cellular STIM1 responsible for activation of the ARC channels is the “minor” pool of STIM1 that is constitutively resident in the plasma membrane. Moreover, the 2 pools of cellular STIM1 respectively located in the ER membrane and the plasma membrane appear to be functionally distinct.24 This raises the possibility that the overall ARC channel activity might be profoundly influenced by processes modulating the relative distribution of the total cellular STIM1 between the ER and plasma membranes. Critically, because the pool of STIM1 involved in ARC channel activation represents only some 15–25% of the total cellular STIM1, even a small redistribution of STIM1 from the ER pool to the PM pool would likely have a profound effect on ARC channel activity. For example, a redistribution of only 5% of the ER pool of STIM1 to the plasma membrane would be expected to represent an approximate 25% increase in the STIM1 available for ARC channel activation. As yet, the factors that are critical in determining the relative distribution of STIM1 to the plasma membrane are currently unclear.

Two important points arise from the findings made above. The first relates to the frequent assumption that the demonstrated role of Orai1 and/or STIM1 in any particular physiological or pathological effect or process necessarily implies that CRAC channels are involved. The problem with this is that these molecules are not unique to the CRAC channels, and such involvement could equally suggest a role for the ARC channels. This can be illustrated by examination of the effects of expression of Orai1 bearing the R91W mutation. Identification of this mutation as the cause of a severe combined immunodeficiency (SCID) resulting from the loss of effective CRAC channel activity in T lymphocytes, played a critical role in the original identification of Orai1 as the protein that formed the CRAC channel.42 Consequently, as shown in Figure 7, expression of this mutant Orai1 in HEK cells stably expressing STIM1 reduced CRAC channels currents by approximately 90% when compared with cells expressing the wild-type Orai1. However, expression of this same mutant Orai1 in these cells also reduced ARC channel currents, by more than 80%. Of course, the essential role of the loss of CRAC channel function in the SCID condition is well established so, in this admittedly simplistic example, we are not suggesting that these data suggest any involvement of the ARC channels in this disease. However, they do indicate how the current emphasis on Orai1 and STIM1 as components of the CRAC channels could lead to erroneous conclusions.

In the same context, an additional point worthy of mention arising from the above findings relates to current programs seeking clinically-relevant compounds that target these proteins. Such programs are of great potential importance, given the
demonstrated role of CRAC channel activity in, among other things, a range of critical immune responses and allergic reactions.\textsuperscript{44,45} However, to date, such programs have focused on Orai1 and, to a lesser extent, STIM1 as potential targets and, as discussed, these molecules are not unique to the CRAC channels. Consequently, targeting these molecules would be expected to impact the ARC channels as much as the CRAC channels. The key issue is that, as yet, the potential physiological, or pathological, roles played by the ARC channels in different tissues are only just beginning to be revealed. However, their widespread expression in various cell types—frequently co-existing with the CRAC channels—clearly suggests that such unique roles exist.

One example of just such a role that has been demonstrated is the ability of calcium entering exclusively via the ARC channels, to modulate the frequency of agonist-induced calcium oscillations as a result of a direct, localized activation of a calcium-dependent phospholipase-C (PLC)\textsuperscript{46} (Fig. 8). Using a sensitive assay for membrane PtdIns(4,5)\textsubscript{P}\textsubscript{2} depletion based on the decline in currents through an expressed Kir2.1 potassium channel, experiments showed that calcium entering via the activated ARC channels induced a highly-localized activation of a PLC\textsubscript{δ} enzyme (PLC\textsubscript{δ}3 in the case of the HEK cells used in the study), resulting in the hydrolysis of membrane PtdIns(4,5)\textsubscript{P}\textsubscript{2} and the generation of InsP\textsubscript{3}. That this effect was exclusive to the ARC channels.

Figure 6. STIM1 in the plasma membrane is constitutively associated with the Orai3 subunits within the ARC channel. (A) Representative western blots showing that STIM1 co-immunoprecipitates with Orai3 (top panel), and that this is not affected by activation of the ARC channels by exogenous arachidonic acid (AA, bottom panel). (B) Representative images showing the co-localization of an eGFP-tagged Lck-STIM1-C construct and a mCherry-tagged Orai3. (C) Representative bimolecular fluorescent complementation image of a cell co-expressing a Lck-STIM1-C construct bearing a C-terminal tag comprised of residues 1–158 of eYFP, and an Orai3 construct N-terminally tagged with the complimentary 159–238 eYFP sequence. (D) Representative images from a FRET experiment showing cells co-expressing a C-terminally tagged Lck-STIM1–C construct (left panel) and a N-terminally tagged Orai3 construct (middle panel), and the resulting FRET image (right panel). The bottom row is a similar FRET experiment except the Lck-STIM1–C construct used (Lck-STIM1–C2A) bore three mutations (W430A, I433A, and L436A) that prevent STIM1 dimerization, resulting in the complete loss of the ability of AA to activate the channels. Data taken from reference 24.
was confirmed by the fact that neither maximal activation of the co-existing CRAC channels, nor increasing global cytosolic calcium concentrations within the normal physiological range (up to 1.3 μM), was able to induce such an increase in PLC activity. Confirmation of the specific role of PLCδ was demonstrated by siRNA knockdown, and by the expression of a lipase-impaired mutant version of the enzyme. The potential physiological relevance of this highly-localized, ARC channel-specific, generation of InsP₃, was revealed by expression of a dominant-negative Orai3 mutant (Orai3-E81Q), or a lipase-impaired PLCδ, either of which reduced the measured rate of ARC channel-induced membrane PtdIns(4,5)P₂ depletion by some 60%, and resulted in an approximate 35% decline in the frequency agonist-induced calcium oscillations. Interestingly, it is possible that this local calcium-induced effect on PLCδ activity might be enhanced by a positive feedback loop. Thus, Missiaen et al., using a membrane vesicle assay, showed that membrane PtdIns(4,5)P₂ acts as an important inhibitory regulator of the plasma membrane calcium pump (PMCA), an action recently confirmed in intact cells by Bandar et al. Thus, the ARC channel-mediated activation of PLCδ would be expected to deplete of PtdIns(4,5)P₂, reducing the activity of the PMCA located close to the channel, thereby further enhancing the calcium-dependent activation of the PLCδ (Fig. 8). Importantly, it is known that the frequency of agonist-induced calcium oscillations is a critical factor in determining the nature of the specific cellular response induced in many different cell types. Consequently, such effects are likely to have widespread physiological significance. Furthermore, the presence of high concentrations of calcium buffers in the cell cytosol imposes significant limits on the diffusional range of any calcium signal. In contrast, despite its much larger size, the functional range of an InsP₃ signal is significantly larger. This may be critical in highly polarized cells such as pancreatic acinar cells, where the calcium release required for exocrine secretion occurs at the apical pole of the cells, whereas the preponderance of agonist receptors are likely located at more distal basal regions of the cell. Finally, an additional point worthy of note is that this ARC channel-mediated, store-independent, generation of InsP₃ might offer an explanation for the commonly observed effect of extracellular calcium on the initial delay, or latency period of calcium responses induced by low agonist concentrations—the time between agonist addition and the first detectible calcium release event. Such an effect is difficult to rationalize if calcium entry was exclusively occurring via the CRAC channels whose activation is dependent on, and subsequent to, calcium release from the ER stores.
Based on the above, it would seem that a key requirement for effectively separating the activities of the ARC channels from the co-existing CRAC channels is a more comprehensive and complete understanding of the precise molecular, structural, and functional features of the ARC channels and their regulation, that distinguish them from the CRAC channels. The studies described above have begun to identify some of these features, but clearly much more needs to be done before the full physiological roles of these channels can be characterized, along with the potential pathological consequences that arise when their activities are disrupted or impaired.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.
36. Korzeniowski MK, Manjarrez IM, Varnai P, Balla T. Activation of STIM1-Orai1 involves an intramolecular switching mechanism. Sci Signal 2010; 3:ra82; PMID:2081754; http://dx.doi.org/10.1126/scisignal.2001122
37. Muik M, Fahner M, Schindl R, Stathopulos P, Frischaufer I, Dreher I, Pfenk P, Lackner B, Groschner K, Ikura M, et al. STIM1 couples to ORAI1 via an intramolecular transition into an extended conformation. EMBO J 2011; 30:1678-89; PMID:21427704; http://dx.doi.org/10.1038/emboj.2011.79
38. Wu MM, Buchanan J, Luik RM, Lewis RS. Ca2+ store depletion causes STIM1 to accumulate in ER regions closely associated with the plasma membrane. J Cell Biol 2006; 174:803-13; PMID:16966422; http://dx.doi.org/10.1083/jcb.200604014
39. Luik RM, Wu MM, Buchanan J, Lewis RS. The elementary unit of store-operated Ca2+ entry: local activation of CRAC channels by STIM1 at ER-plasma membrane junctions. J Cell Biol 2006; 174:815-25; PMID:16966423; http://dx.doi.org/10.1083/jcb.200604015
40. Lur G, Haynes LP, Prior IA, Gerasimenko OV, Ferse S, Petersen OH, Burgoyne RD, Tepikin AV. Ribosome-free terminals of rough ER allow formation of STIM1 puncta and segregation of STIM1 from IP3 receptors. Curr Biol 2009; 19:6648-53; PMID:19765991; http://dx.doi.org/10.1016/j.cub.2009.07.072
41. Orci L, Ravazzola M, Le Coadic M, Shen WW, Demaurex N, Cosson P. From the Cover: STIM1-induced pre cortical and cortical subdomains of the endoplasmic reticulum. Proc Natl Acad Sci U S A 2009; 106:19388-62; PMID:19906089; http://dx.doi.org/10.1073/pnas.0911280106
42. Zhou Y, Srinivasan P, Razavi S, Seymour S, Meraner P, Gudlat A, Stathopulos PB, Ikura M, Rao A, Hogan PG. Initial activation of STIM1, the regulator of store-operated calcium entry. Nat Struct Mol Biol 2013; 20:973-81; PMID:23851458; http://dx.doi.org/10.1038/nsmb.2625
43. Ferse S, Müller JM, Graf D, Kroczyk RA, Dräger R, Niemeyer C, Baeuerle PA, Peter HH, Schlesier M. Severe combined immunodeficiency due to defective binding of the nuclear factor of activated T cells in T lymphocytes of two male siblings. Eur J Immunol 1996; 26:2119-26; PMID:8814256; http://dx.doi.org/10.1002/eji.1830260924
44. Ferse S. CRAC channelopathies. Pfleugers Arch 2010; 460:417-35; PMID:20111871; http://dx.doi.org/10.1007/s00424-009-0777-5
45. Parekh AB. Store-operated CRAC channels: function in health and disease. Nat Rev Drug Discov 2010; 9:399-410; PMID:20395953; http://dx.doi.org/10.1038/nrd3136
46. Thompson JL, Shuttleworth TJ. Orai channel-dependent activation of phospholipase C-δ: a novel mechanism for the effects of calcium entry on calcium oscillations. J Physiol 2011; 589:5057-69; PMID:21878252; http://dx.doi.org/10.1113/jphysiol.2011.214437
47. Missiaen L, Raeymaekers L, Wuystack F, Vrolix M, de Smedt H, Casteels R. Phospholipid-protein interactions of the plasma-membrane Ca2+-transporting ATPase. Evidence for a tissue-dependent functional difference. Biochem J 1989; 263:687-94; PMID:2532005
48. Bandara S, Malmersjo S, Meyer T. Regulators of Calcium Homeostasis Identified by Inference of Kinetic Model Parameters from Live Single Cells. Sci Signal 2013; 6:ra56; PMID:23838183; http://dx.doi.org/10.1126/scisignal.2003649
49. Allbritton NL, Meyer T, Stryer L. Range of messenger action of calcium ion and inositol 1,4,5-trisphosphate. Science 1992; 258:1812-5; PMID:1465619; http://dx.doi.org/10.1126/science.1465619
50. Rooney TA, Sasi EJ, Thomas AP. Characterization of cytosolic calcium oscillations induced by phenylephrine and vasopressin in single fura-2-loaded hepatocytes. J Biol Chem 1989; 264:17131-41; PMID:2793847
51. Berridge MJ. Relationship between latency and period for 5-hydroxytryptamine-induced membrane responses in the Calliphora salivary gland. Biochem J 1994; 302:545-50; PMID:8093009
52. Shuttleworth TJ, Thompson JL. Evidence for a non-capacitative Ca2+ entry during [Ca2+] oscillations. Biochem J 1996; 316:819-24; PMID:8670157