Mechanisms of capacitative calcium entry

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Summary

Capacitative Ca^{2+} entry involves the regulation of plasma membrane Ca^{2+} channels by the filling state of intracellular Ca^{2+} stores in the endoplasmic reticulum (ER). Several theories have been advanced regarding the mechanism by which the stores communicate with the plasma membrane. One such mechanism, supported by recent findings, is conformational coupling: inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3) receptors in the ER may sense the fall in Ca^{2+} levels through Ca^{2+}-binding sites on their lumenal domains, and convey this conformational information directly by physically interacting with Ca^{2+} channels in the plasma membrane. In support of this idea, in some cell types, store-operated channels in excised membrane patches appear to depend on the presence of both Ins(1,4,5)P_3 and Ins(1,4,5)P_3 receptors for activity; in addition, inhibitors of Ins(1,4,5)P_3 production that either block phospholipase C or inhibit phosphatidylinositol 4-kinase can block capacitative Ca^{2+} entry. However, the electrophysiological current underlying capacitative Ca^{2+} entry is not blocked by an Ins(1,4,5)P_3 receptor antagonist, and the blocking effects of a phospholipase C inhibitor are not reversed by the intracellular application of Ins(1,4,5)P_3. Furthermore, cells whose Ins(1,4,5)P_3 receptor genes have been disrupted can nevertheless maintain their capability to activate capacitative Ca^{2+} entry channels in response to store depletion. A tentative conclusion is that multiple mechanisms for signaling capacitative Ca^{2+} entry may exist, and involve conformational coupling in some cell types and perhaps a diffusible signal in others.

Key words: Calcium channels, Capacitative calcium entry, Calcium signaling, Signal transduction

Introduction

The process of cellular Ca^{2+} signaling involves regulated changes in the concentration of Ca^{2+} in the cytoplasm ($[Ca^{2+}]_i$) and other cellular compartments. A multitude of cellular processes are controlled through Ca^{2+} signaling and, in turn, a multitude of external cellular signals induce or regulate Ca^{2+} signaling. Because so many systems respond to, or regulate, Ca^{2+} signaling, it is not surprising that dysfunctions of various aspects of Ca^{2+} signaling pathways underlie several important diseases (Missiaen et al., 2000).

When calcium signaling is stimulated in a cell, Ca^{2+} enters the cytoplasm from one of two general sources: it is released from intracellular stores, or it enters the cell across the plasma membrane. Both processes often occur either simultaneously or sequentially. In many excitable cells, entry of Ca^{2+} can be activated by membrane depolarization. This signal is then amplified as the entering Ca^{2+} triggers additional mobilization of intracellular Ca^{2+} stores through activation of ryanodine receptor channels, a process known as Ca^{2+}-induced Ca^{2+} release. In some excitable cells and, with the exception of mammalian erythrocytes, essentially all non-excitable cells, an important initiating step is intracellular release of Ca^{2+} from internal stores by binding of a second messenger to its receptor in the endoplasmic reticulum (ER). Commonly, this messenger is inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3; Berridge, 1993), but a number of other potential messengers have been discovered in recent years (Lee et al., 1999; Petersen and Cancela, 1999). Ca^{2+} entry can be signaled by a variety of processes, including direct activation by surface receptors and activation by a variety of second messengers (Clementi and Meldolesi, 1996). However, the most commonly observed mechanism of regulated Ca^{2+} entry in non-excitable cells is a process know as *capacitative Ca^{2+} entry* or *store-operated Ca^{2+}* entry (Putney, 1986; Putney, 1997). In this mechanism, the depletion of intracellular stores due to the action of Ins(1,4,5)P_3 or other Ca^{2+}-releasing signals activates a signaling pathway leading to the opening of plasma membrane Ca^{2+} channels (Fig. 1). Several recent reviews (Berridge, 1995; Parekh and Penner, 1997; Barritt, 1999) and a monograph (Putney, 1997) have dealt with capacitative Ca^{2+} entry from a variety of perspectives. In recent years, considerable attention has focused on the nature of the signal linking intracellular Ca^{2+} stores to capacitative Ca^{2+} entry. Here, we summarize some of the recent findings and theories and attempt a critical assessment of one currently popular idea, the conformational coupling model.

Proposals for coupling mechanisms

Perhaps the simplest mechanism for linking intracellular stores to the plasma membrane is through the release of a diffusible messenger (Fig. 1A). This idea was perhaps the first possibility to be put forth in print (Putney, 1990), but the first experimental evidence for such a messenger was provided by Randriamampita and Tsien (Randriamampita and Tsien, 1993). They described an activity in extracts of stimulated Jurkat T cells that increased Ca^{2+} entry in several cell types. This activity they termed CIF (for calcium influx factor). Further fractionation and purification of cell extracts produced an activity, probably different from the first CIF described by

Randriamampita and Tsien, that potently activated Ca^{2+} influx when injected into *Xenopus* oocytes (Kim et al., 1995) or when applied to patches containing excised, store-operated channels (Thomas et al., 1996; Csutora et al., 1999; Trepakova et al., 2000). The most recently published data on CIF action present a compelling case for such a molecule (Csutora et al., 1999; Trepakova et al., 2000). There are two problems with the current data on CIF: (1) CIF appears to be relatively stable when injected into oocytes, even at submaximally effective levels; and (2) the channels activated by CIF in oocytes are relatively lanthanum insensitive, whereas the endogenous store-operated channels are completely inhibited by lanthanum (Csutora et al., 1999).

Two alternative hypotheses involve more direct, physical interactions between the plasma membrane and underlying structures. One idea – the exocytosis model (Fig. 1B) originally suggested by Fasolato et al. – is that channels may be inserted into the membrane by vesicle fusion in response to store depletion (Fasolato et al., 1993). Yao et al. recently presented considerable molecular evidence in favor of this idea (Yao et al., 1999), and the implications of these findings have been discussed (Putney, 1999). Although this idea provides a mechanism by which the channels become active in the plasma membrane, it does not provide any insight into how the process is coupled to store depletion. The channels in the vesicles and Ins(1,4,5)P_3 receptors might interact physically, as is suggested in the conformational coupling model (Putney, 1999), but other mechanisms, perhaps involving a diffusible signal, are equally possible. The major problem with this suggestion is that exocytosis is thought to be Ca^{2+} dependent. But activation of store-operated entry occurs maximally when $[Ca^{2+}]_i$ is strongly buffered to low levels, and, when $[Ca^{2+}]_i$ is reduced to very low levels, this reduction in $[Ca^{2+}]_i$ is sufficient to activate the channels (Kerschbaum and Cahalan, 1999; Krause et al., 1999; Braun et al., 2001). Also, two studies have demonstrated activation of single, store-operated channels following excision of membrane patches (Zubov et al., 1999; Braun et al., 2001). This suggests that the channels are already present in the cell membrane prior to activation.

Another idea put forth at the initial conception of the capacitative model (Putney, 1986) involves regulation of the channels through control of the concentration of Ca^{2+} in their immediate vicinity (Fig. 1C). It is well established that $[Ca^{2+}]_i$ in the vicinity of the plasma membrane channels inhibits the channels (Poggioli et al., 1985; Krause et al., 1999). Thus, depletion of stores might result in a fall in $[Ca^{2+}]_i$ in a small, restricted space between the plasma membrane and closely associated ER, which would activate the channels. This idea was resurrected in a review by Barritt (Barritt, 1998). More recent electrophysiological studies have clearly demonstrated high sensitivity of the store-operated channels to inhibition by $[Ca^{2+}]_i$ (Parekh and Penner, 1997), and Krause et al. have shown that simply lowering $[Ca^{2+}]_i$ in the cell causes full activation of a well-characterized current reflecting capacitative Ca^{2+} entry known as I_{crac} (for calcium release-activated calcium current; Hoth and Penner, 1992; Krause et al., 1999). There are two major problems with this idea. The first is the ability of store depletion to activate I_{crac} in the face of substantial cytoplasmic Ca^{2+} buffering (Parekh and Penner, 1997). This objection could possibly be overcome if the distance between the source of Ca^{2+}, presumably the subsurface ER, and the plasma membrane channels is limited. The second problem is the ability of the ER Ca^{2+} pump inhibitor, thapsigargin, to activate I_{crac} (and other instances of capacitative Ca^{2+} entry). The published models for a Ca^{2+}-regulated mechanism (Putney, 1986; Barritt, 1998) have assumed that $[Ca^{2+}]_i$ in the restricted space near the channels is regulated by a balance of leakage from the ER and uptake by the ER Ca^{2+} pumps. Treatment with thapsigargin would prevent the underlying ER from lowering $[Ca^{2+}]_i$ near the channels, and activation would not be expected to occur. However, Ca^{2+} pumps need not be involved in the restricted space. Rather, the key element may simply be the input of Ca^{2+} into this region by a replete ER pool. This input would occur through channels of an unknown nature, presumably those that mediate the constant passive leakage of Ca^{2+} out of the ER. Ca^{2+} removal from the space could be accomplished either by mobile buffers or by nearby plasma membrane pumps, which are not thapsigargin sensitive.

Conformational coupling

At present, the favored model for capacitative Ca^{2+} entry is conformational coupling (Fig. 1D). Irvine first described a mechanism of communication between the ER and the plasma membrane that involves a direct protein-protein interaction rather than a diffusible messenger (Irvine, 1990). He noted that, in the case of skeletal muscle, plasma membrane L-type voltage-dependent Ca^{2+} channels communicate with intracellular Ca^{2+}-release channels, in this case ryanodine receptors, through a direct protein-protein interaction. In the case of skeletal muscle, information flows from the Ca^{2+} channel in the t-tubule membrane to the ryanodine receptor (Ebashi, 1991), whereas for capacitative Ca^{2+} entry the flow of information would be in the opposite direction. A detailed analysis of this proposal has been presented by Berridge (Berridge, 1995). Several recent studies have now provided support for such a mechanism, at least in specific experimental systems.

A brief digression is necessary at this point to discuss a family of proteins that have been considered as candidates for capacitative Ca^{2+} entry channel components. These are the mammalian homologs of *Drosophila* TRP (for transient receptor potential), a component of a cation channel in the *Drosophila* photoreceptor that is activated by a poorly understood mechanism involving phospholipase C. Seven mammalian TRPs (TRP1-TRP7, also known as TRPC1-TRPC7) are reasonably closely related to *Drosophila* TRP, and to one another (~30-80% sequence identity; Putney and McKay, 1999; Harteneck et al., 2000). In some instances, the overexpression of a specific TRP has been shown to augment capacitative Ca^{2+} entry (Philipp et al., 1996; Zitt et al., 1996; Kiselyov et al., 1998; Liu et al., 2000), but in many instances these findings have been difficult to reproduce in other laboratories (Zhu et al., 1998; Schaefer et al., 2000; McKay et al., 2000). Nevertheless, there is general agreement from several studies that TRP3 (and probably its close relatives, TRP6 and TRP7) can be activated in an Ins(1,4,5)P_3- and Ins(1,4,5)P_3-receptor-dependent manner (Zhu et al., 1996; Zhu et al., 1998; Kiselyov et al., 1998; McKay et al., 2000). Thus, TRP3 has been investigated as a model for understanding conformational coupling, although its identity as a component

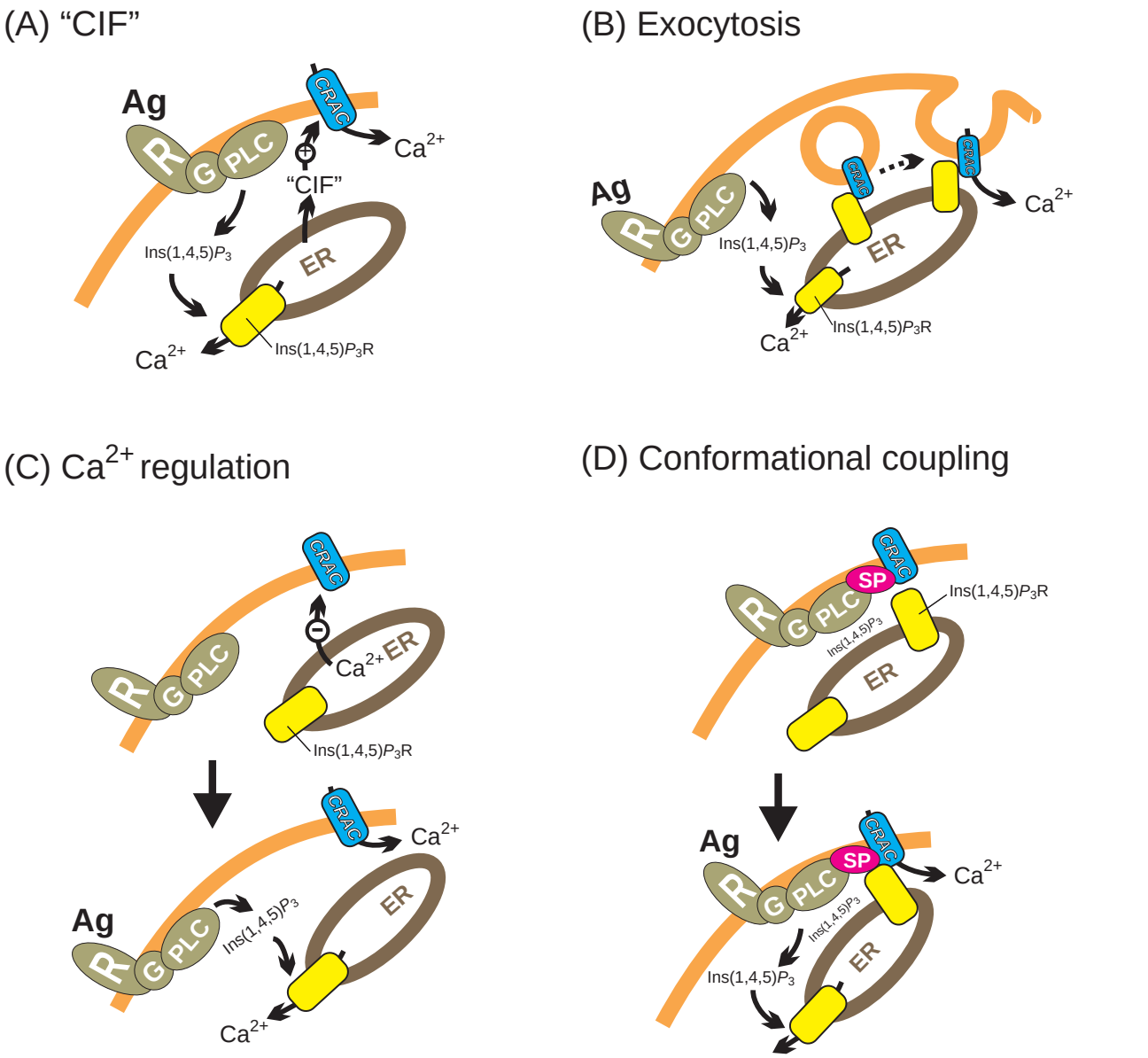

Fig. 1. Proposed mechanisms for signaling capacitative calcium entry. In all models, agonist activation results in the production of Ins(1,4,5)P_3, which results in discharge of stored Ca^{2+}. Alternatively, Ca^{2+} stores can be discharged passively through the use of reagents such as thapsigargin or ionomycin (not shown). (A) Following discharge of the stores, a diffusible signal – Ca^{2+}-influx factor (CIF) – is released from the ER and activates plasma membrane store-operated channels. (B) In the exocytosis model, depletion of stores (in this depiction, through something similar to the conformational coupling model) causes fusion of vesicles containing CRAC channels with the plasma membrane. (C) In the Ca^{2+} regulation model, Ca^{2+} discharged from a replete Ca^{2+} pool keeps the channels in an inhibited state. Discharge of the stores removes the source of this inhibitory Ca^{2+} and relieves the inhibition. (D) In the conformational coupling model, discharge of Ca^{2+} stores leads to a conformational change in the Ins(1,4,5)P_3 receptor, which is transmitted to plasma membrane Ca^{2+} channels by a direct protein-protein interaction. Abbreviations: R, agonist receptor; Ag, agonist; G, heterotrimeric G protein; PLC, polyphosphoinositide phospholipase C; CRAC, Ca^{2+} release-activated Ca^{2+} (channel); ER, endoplasmic reticulum; Ins(1,4,5)P_3, inositol 1,4,5-trisphosphate; Ins(1,4,5)P_3R, Ins(1,4,5)P_3 receptor; CIF, Ca^{2+}-influx factor; SP, scaffolding protein.

of a capacitative Ca^{2+} entry channel has not been fully established.

The first evidence that Ins(1,4,5)P_3 might play a role in capacitative Ca^{2+} entry came from a study of store-operated channels in endothelial cells. Vaca and Kunze identified small single channels in endothelial cells that were activated by depletion of intracellular Ca^{2+} stores by a Ca^{2+}-pump inhibitor (Vaca and Kunze, 1994; Vaca and Kunze, 1995). When excised, channel activity decreased rapidly but could be restored by addition of Ins(1,4,5)P_3. Subsequently, Kiselyov et al. described similar behavior of agonist-activated TRP3 channels in HEK-293 cells stably transfected with this *trp3* (Kiselyov et al., 1998). After more prolonged perifusion of the excised patches, the ability to restore activity by addition of Ins(1,4,5)P_3 was lost, and the addition of Ins(1,4,5)P_3 receptor together with Ins(1,4,5)P_3 was required. In a subsequent study,

Kiselyov et al. demonstrated that the minimal portion of the Ins(1,4,5)P_3 receptor required to activate the TRP3 channels was the N-terminal Ins(1,4,5)P_3-binding domain (Kiselyov et al., 1999). Zubov et al. demonstrated a similar dependence of endogenous store-operated channels on Ins(1,4,5)P_3 and Ins(1,4,5)P_3 receptors in A431 cells (Zubov et al., 1999). These results provide strong evidence for a functional interaction between both TRP3 and capacitative Ca^{2+} entry channels with Ins(1,4,5)P_3-liganded Ins(1,4,5)P_3 receptors. Biochemical evidence consistent with this conclusion was provided by the demonstration that expressed, tagged TRP3 can be co-immunoprecipitated with Ins(1,4,5)P_3 receptors (Kiselyov et al., 1999; Boulay et al., 1999). Finally, Boulay et al. used a GST-pull-down strategy to map the TRP3-Ins(1,4,5)P_3 receptor interacting sequences and showed that expression of these peptides modulates endogenous capacitative Ca^{2+} entry (Boulay et al., 1999).

Collectively, these findings provide strong support for the conformational coupling hypothesis. The data have two significant implications. (1) In addition to a requirement for the Ins(1,4,5)P_3 receptor, surprisingly there appears to be a requirement for Ins(1,4,5)P_3. This implies a requirement for the system that makes Ins(1,4,5)P_3, phospholipase C and its substrate phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2). (2) Despite the demonstration that in excised patches Ins(1,4,5)P_3, and an Ins(1,4,5)P_3 receptor or Ins(1,4,5)P_3-binding domain can activate the channel, in situ the controlling factor is clearly the filling state of the intracellular Ca^{2+} stores. Thus, one must propose that the Ins(1,4,5)P_3-liganded form of the receptor is in an activating configuration, unless the C-terminal lumenal domain is exposed to Ca^{2+} in the fully loaded ER of unstimulated cells.

The inclusion of Ins(1,4,5)P_3 in the conformation coupling model presents some problems given the clear demonstration by many studies that activation of PLC is not required for full activation of capacitative Ca^{2+} entry – i.e. when agents such as thapsigargin or ionomycin are used to deplete the stores. One possible resolution of this problem would be a situation in which the store-operated channels and associated Ins(1,4,5)P_3 receptor(s) are distributed in very close proximity to PLC molecules, perhaps through an organized signaling complex (Putney, 1999). This would be analogous to the situation in *Drosophila* photoreceptors, in which PLC is complexed with TRP and other signaling molecules through a scaffolding protein, INAD (Huber et al., 1996; Li and Montell, 2000).

We used a pharmacological approach to investigate the potential roles of PLC and Ins(1,4,5)P_3 receptors in activation of capacitative Ca^{2+} entry and I_{crac} (Broad et al., 2001). U73122, a membrane-permeant inhibitor of phospholipase C (Smith et al., 1990), inhibits capacitative Ca^{2+} entry (Berven and Barritt, 1995), although this was considered to be a side effect of the drug probably unrelated to its action against phospholipase C. We found that U73122 inhibits capacitative Ca^{2+} entry in epithelial cells and in the mast cell line RBL and also completely blocks I_{crac} in RBL cells (Broad et al., 2001). Furthermore, this inhibitory action appears to result from inhibition of PLC. We also demonstrated that depletion of cellular polyphosphoinositides by the phosphatidylinositol 4-kinase inhibitor wortmannin completely blocks store-operated entry; Rosado and Sage previously reported similar findings (Rosado and Sage, 2000b). These results are consistent with the suggestion that a basal phospholipase C activity, which produces Ins(1,4,5)P_3 from the precursor lipid, PtdIns(4,5)P_2, is required for capacitative Ca^{2+} entry. In support of this idea, the membrane-permeant Ins(1,4,5)P_3 receptor antagonist 2-aminoethoxydiphenyl borane (2APB; Maruyama et al., 1997) completely blocks activation of TRP3 channels and capacitative Ca^{2+} entry (Ma et al., 2000), as well as I_{crac} (Braun et al., 2001). However, in the case of I_{crac}, the block by 2APB appears to result from a more direct effect on the channels and does not involve the Ins(1,4,5)P_3 receptor (Braun et al., 2001). Furthermore, neither capacitative Ca^{2+} entry in lacrimal cells nor I_{crac} in RBL cells is blocked by intracellular application of the competitive Ins(1,4,5)P_3 receptor antagonist heparin (Bird et al., 1991; Broad et al., 2001). Finally, we found that the inhibition of I_{crac} by phospholipase C inhibition, or by depletion of PtdIns(4,5)P_2 cannot be reversed by intracellular addition of Ins(1,4,5)P_3 (Broad et al., 2001).

Particularly troublesome for the conformational coupling model is the behavior of a DT40 pre-B-lymphocyte cell line from which all three Ins(1,4,5)P_3 receptor types were eliminated by targeted gene disruption (Sugawara et al., 1997). These cells lose all ability to respond to phospholipase-C-coupled agonists, or to intracellular application of Ins(1,4,5)P_3, but give normal capacitative Ca^{2+} entry responses to thapsigargin (Sugawara et al., 1997; Broad et al., 2001). In addition, both the wild-type and Ins(1,4,5)P_3-receptor-knockout DT40 cells show no capacitative Ca^{2+} entry response following depletion of PtdIns(4,5)P_2 by wortmannin. Kiselyov et al. have argued that the site of gene disruption in the DT40 cells is close to the C-terminus (Kiselyov et al., 1998), and this might result in the expression of a truncated, Ins(1,4,5)P_3 binding protein. Although such a protein would probably not function as an Ins(1,4,5)P_3-gated channel, it might retain the ability to regulate TRP or store-operated channels. In support of this idea, Kiselyov et al. expressed such a truncated Ins(1,4,5)P_3 receptor and demonstrated that it can support TRP3 channel activity in excised patches as effectively as the complete Ins(1,4,5)P_3 receptor (Kiselyov et al., 1998). In light of this finding, we looked for specific Ins(1,4,5)P_3-binding sites in both wild-type and Ins(1,4,5)P_3-receptor-knockout DT40 cells. Membranes from wild type cells contained ~17 fmol/mg protein binding sites, whereas no significant binding of Ins(1,4,5)P_3 could be detected in the knockout cells (Broad et al., 2001). This indicates that despite the theoretical argument advanced by Kiselyov et al. (Kiselyov et al., 1998), the gene-disrupted cells do not express significant amounts of a truncated Ins(1,4,5)P_3-binding protein. Thus, for the DT40 cells, as for the RBL cells, whereas PtdIns(4,5)P_2 and PLC appear to be required for coupling store depletion to Ca^{2+} entry, there does not appear to be a role for Ins(1,4,5)P_3 or Ins(1,4,5)P_3 receptors. One report has suggested that ryanodine receptors can also couple to the channels in the same manner as Ins(1,4,5)P_3 receptors (Kiselyov et al., 2000); however, neither wild-type nor Ins(1,4,5)P_3-receptor-knockout DT40 cells respond to caffeine, which suggests that few if any ryanodine receptors are expressed in these cells.

Even if ryanodine receptors were able to gate the channels, this does not help with the question of why PLC is needed. Two obvious possibilities are a requirement for the other product of PtdIns(4,5)P_2 hydrolysis, diacylglycerol (DG), or a requirement for a decrease in the level of PtdIns(4,5)P_2 at a

critical site. DG does not seem to be required, because the inhibitory effects of U73122 were not relieved by exogenous addition of a membrane-permeant diacylglycerol, 1-oleoyl-2-acetyl-*sn*-glycerol (OAG; Broad et al., 2001). A decrease in PtdIns(4,5)P_2 does not seem to be important, because depletion of PtdIns(4,5)P_2 by inhibition of PI kinase actually blocks activation. Thus, the role of PLC is uncertain. The situation may be analogous to that in the *Drosophila* photoreceptor, in which PLC (norpA) is required for signaling (Bloomquist et al., 1988), but the Ins(1,4,5)P_3 receptor is not (Acharya et al., 1997). Similarly, PtdIns(4,5)P_2 appears to be necessary but not as a precursor for Ins(1,4,5)P_3 or DG. PtdIns(4,5)P_2 has multiple roles in signal transduction (Martin, 1998) and is important in attachment and function of cytoskeletal elements at the plasma membrane. Rosado and Sage have suggested that the role of PtdIns(4,5)P_2 involves the cytoskeleton (Rosado and Sage, 2000b; Rosado and Sage, 2000c). However, published results on the effects of disruption of the cytoskeleton on capacitative Ca^{2+} entry have yielded conflicting results (Holda and Blatter, 1997; Ribeiro et al., 1997; Patterson et al., 1999).

Reconciliation?

How can we reconcile two such strong bodies of evidence – one favoring conformational coupling of store-operated channels to Ins(1,4,5)P_3 receptors, and the other refuting this idea? It may be useful to look at the original idea of conformational coupling proposed by Irvine (Irvine, 1990). The model upon which Irvine based his thinking was the coupling of L-type Ca^{2+} channels in the t-tubule membrane of skeletal muscle cells to ryanodine receptor Ca^{2+} release channels in the sarcoplasmic reticulum. For this system, the evidence favoring conformational coupling is very solid. Yet, on this basis, would we propose that communication between voltage-dependent Ca^{2+} channels and ryanodine receptors occurs by a similar mechanism throughout the body? Taking the case of the heart as a well-studied example, here it is clear that the same signaling outcome is achieved – coupling of Ca^{2+} channel activation to ryanodine receptor activation – and yet, in this instance, it is clear that the signal is not a conformational one but a diffusible one – Ca^{2+} activates the Ca^{2+}-induced Ca^{2+} release response of the ryanodine receptor (Fabiato, 1983). Thus, despite the appropriateness of the skeletal muscle arrangement as a basis for the conformational coupling model, it is clear that, for the ryanodine receptor, this model applies probably only in one very specific situation; in most if not all other situations, the signaling is via a diffusible messenger – in this case, Ca^{2+} itself.

It is therefore not so difficult to accept the possibility that multiple mechanisms can regulate store-operated channels. The two cell types for which there is the best direct experimental evidence for Ins(1,4,5)P_3-receptor-mediated, conformational coupling to endogenous, store-operated channels are endothelial cells (Vaca and Kunze, 1994) and A431 cells (Zubov et al., 1999)[1]. Note that these two cell types are very similar in their electrophysiological behavior and are quite distinct from the more extensively investigated blood cells, which show the classical I_{crac} response to store depletion. Specifically, the single-channel conductances in endothelial cells and A431 cells are much greater, and the permeability of the channels to divalent cations other than Ca^{2+} is much greater than that of the channels underlying I_{crac} (Hoth and Penner, 1992; Zweifach and Lewis, 1993; Lückhoff and Clapham, 1994; Vaca and Kunze, 1994). Thus, we may tentatively conclude that the conformational coupling model remains a tenable hypothesis for certain cell types but may not necessarily apply in all cases.

What are the most likely mechanisms for activation of store-operated channels in cells that do not utilize conformational coupling? Once we allow for different mechanisms in different cell types, no one idea can be unequivocally ruled out. As stated above, the CIF concept remains a viable alternative, and the idea of localized $[Ca^{2+}]_i$ buffering deserves serious consideration. Although there are clear instances in which the exocytosis model seems unlikely, there may also be situations in which such a mechanism operates. And finally, we cannot rule out mechanisms combining these concepts: a diffusible signal required for conformational coupling to take place, or a need to reduce $[Ca^{2+}]_i$ under the channels before Ins(1,4,5)P_3 receptors can interact, or a conformational coupling-exocytosis model (as drawn in Fig. 1B).

Conclusions

In recent years, much research has focused on the mechanism linking depletion of intracellular Ca^{2+} stores to the activation of plasma membrane Ca^{2+} channels. There is considerable evidence for a conformational coupling mechanism by which depletion of intracellular Ca^{2+} stores induces a conformational change in an Ins(1,4,5)P_3 receptor, which, in an Ins(1,4,5)P_3-dependent manner, interacts with and activates a plasma membrane Ca^{2+} channel. However, there is also a convincing body of evidence indicating that such a mechanism cannot account for the activation of store-operated channels in all instances. Other possibilities include the generation of a diffusible Ca^{2+}-influx factor and activation by relief of a tonic Ca^{2+}-dependent inhibition of the channels (see Fig. 1). Clearly much still needs to be done if we are to sort out these possibilities and better understand the regulation of this major Ca^{2+} signaling pathway.

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