Mini Review

Mechanism of store-operated calcium entry

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Activation of receptors coupled to the phospholipase C/IP_3 signalling pathway results in a rapid release of calcium from its intracellular stores, eventually leading to depletion of these stores. Calcium store depletion triggers an influx of extracellular calcium across the plasma membrane, a mechanism known as the store-operated calcium entry or capacitative calcium entry. Capacitative calcium current plays a key role in replenishing calcium stores and activating various physiological processes. Despite considerable efforts, very little is known about the molecular nature of the capacitative channel and the signalling pathway that activates it. This review summarizes our current knowledge about store operated calcium entry and suggests possible hypotheses for its mode of activation.

1. Introduction

Calcium is a crucial regulator of many physiological processes, and a variety of stimuli produce their cellular effects by increasing the cytosolic free calcium concentration ($[Ca^{2+}]_i$). This increase in $[Ca^{2+}]_i$ can be due to calcium entry from the extracellular space, through channels in the plasma membrane or from intracellular stores of calcium (mainly endoplasmic reticulum, ER). In a variety of electrically non-excitable cells (for example epithelial cells, blood cells, fibroblasts), and in many instances for excitable cells, agonists of G-protein linked receptors activate phospholipase C (PLC) causing hydrolysis of phosphatidylinositol (4,5) biphosphate (PIP_2) to release the signalling molecule inositol-1,4,5-trisphosphate (IP_3). The receptor for IP_3 (IP_3R) is located on the membrane of the internal stores and functions as a ligand-gated channel. Its activation by IP_3 leads to a rapid release of calcium in the cytoplasm resulting in an increase in $[Ca^{2+}]_i$.

An exciting development came with the discovery that in many non-excitable cells, the depletion of internal calcium stores triggers an influx of calcium across the plasma membrane (see review Putney and McKay 1999). This form of calcium entry was termed as store-operated calcium entry (also known as capacitative calcium entry) (see figure 1). Experimentally, this calcium influx can be activated by agents that deplete calcium stores, such as thapsigargin (which is a potent inhibitor of Ca^{2+}-ATPases). Proposed functions of this mechanism of calcium entry are to replete the internal stores and to play an important role in various other physiological processes (such as T-cell activation, mast cell degranulation) by prolonging the increased cytosolic calcium concentration. The capacitative model of calcium entry has been confirmed in a large number of cell types and is clearly a widespread mechanism of calcium entry.

Work in this area has proceeded mainly along two paths. First, to identify the protein channel (commonly referred to as the store operated calcium channel or SOC) that serves this function. Second, to delineate the mechanism by which the signal is transmitted from the depleted stores to the SOCs in the plasma membrane. This review focuses primarily on the research that has been carried out on these two aspects of store operated calcium entry.

2. Molecular identity of the SOCs

Before discussing the molecular candidates for capacita-

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Abbreviations used: ER: Endoplasmic reticulum; PLC: phospholipase C; PIP: phosphatidylinositol (4,5) biphosphate; IP_3: inositol-1,4,5-trisphosphate; SOC: store operated calcium channel; TRP: transient receptor potential; CIF: calcium influx factor; DHPR: dihydropyridine receptor.

tive calcium entry channels, we should consider some of the properties of such channels. At present the best characterized capacitative current is the calcium release-activated calcium current (I_{CRAC}), which has a very low conductance (~ 20 femtoSiemens), extremely high selectivity for calcium over monovalent ions and a strong negative feedback regulation by calcium (Berridge 1995). Other types of capacitative calcium entry current have also been observed in endothelial cells, A431 cells and mouse pancreatic acinar cells (see review by Berridge 1995). But these currents differ from I_{CRAC} in terms of higher unitary conductance and a substantially higher preference for other cations like Ba^{2+}. For detailed information about the kinetics of capacitative calcium entry, one can refer to the review by Berridge (1995).

Central to resolving the issue of capacitative calcium entry was the molecular identification of the channel protein. Major excitement ensued from the isolation of two candidate proteins from *Drosophila* – the transient receptor potential (TRP) and its related congener, TRPL. *trp* is a *Drosophila* photoreceptor mutant incapable of maintaining a sustained receptor potential in response to photostimulation, thus showing its role in phototransduction (Hardie and Minke *et al* 1992). *trp* was cloned and found to have a weak homology with the mammalian voltage dependent calcium channels (Phillips *et al* 1992). The idea that TRP could function as a capacitative calcium entry channel originated with studies of Schilling's group and later on substantiated by others (Vaca *et al* 1994). They found that expression of TRP in insect Sf9 cells or *Xenopus* oocytes results in a TRP-dependent conductance that can be activated by thapsigargin-induced calcium store depletion. Schilling and coworkers also provided evidence that the sensitivity of TRP to store depletion resides somewhere in its rather lengthy C-terminal cytoplasmic region which contains an unusual proline rich domain (Sinkins *et al* 1996). Even though *Drosophila* TRP has been found to conduct capacitative calcium current in vertebrate cell culture systems, it is becoming increasingly clear that TRP does not function as an SOC in *Drosophila* photoreceptors. A comparison of the biophysical properties of TRP and TRPL with I_{CRAC} shows clear differences (Bennett *et al* 1995). *Drosophila* photoreceptors lacking IP_3 show normal visual transduction and normal TRP function, indicating that IP_3 mediated store depletion does not underlie TRP activation (Acharya *et al* 1997; Raghu *et al* 2000).

However, discovery of TRP and its possible role as an SOC resulted in a hunt for similar proteins in vertebrates and the results were soon very positive. Several laboratories reported the successful cloning of TRP homologues in human, mouse, rat, rabbit, bovine and *Xenopus*. Addi-

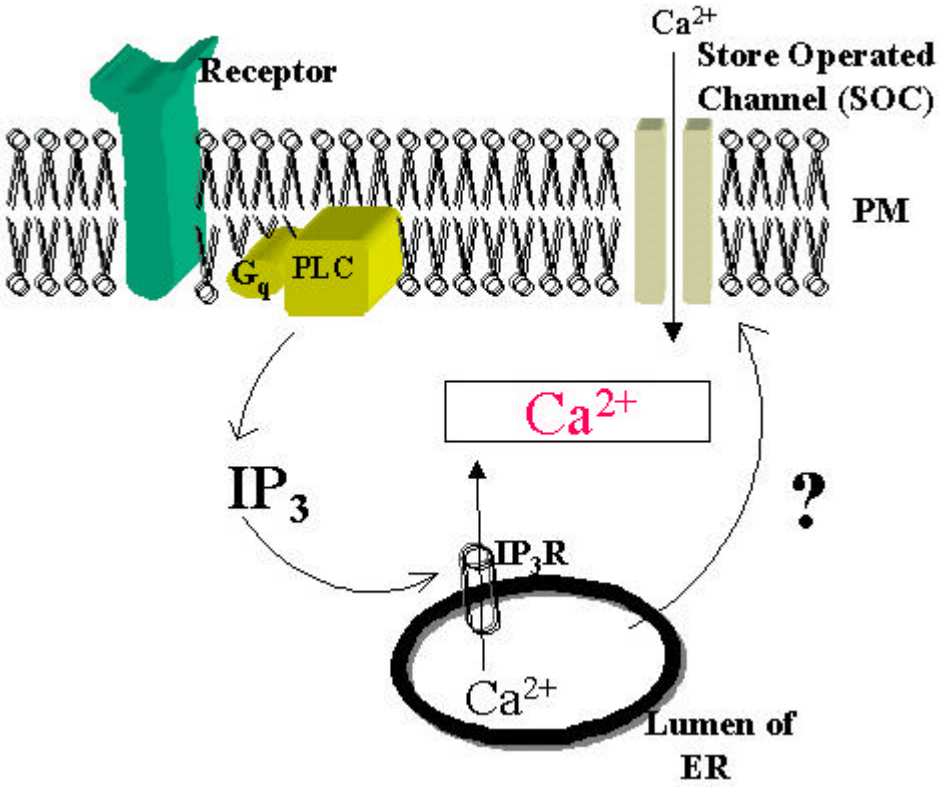

Figure 1. Capacitative calcium entry.

tionally, thirteen members of the *Caenorhabditis elegans* TRP family of proteins have been predicted (for detailed references see the reviews by Putney and McKay 1999 and by Harteneck *et al* 2000). There are at present seven known mammalian TRP forms (designated TRP1 to TRP7) and many more may be discovered. The structures of these TRP proteins, based on their complete amino acid sequence, have been predicted (Putney and McKay 1999). The common structural features include six transmembrane spanning region and four ankyrin repeats in the N-terminal region.

For the vertebrate TRP homologues to be candidate SOCs, a more stringent requirement would be their conductance behaviour. Most of these TRPs were expressed in heterologous expression systems and their ion conducting properties assayed. Table 1 summarizes the results obtained from the expression of the different TRP homologues. There appears no clear consensus regarding the function of these homologues. A few of the proteins function as nonselective cation channel while the rest function as calcium channels, some activated by agonists while others activated by store depletion. Even in the same group (like TRP4) proteins of different species show contradictory results in terms of SOC function. In cases where the protein does function as an SOC, the current never matches exactly with I_{CRAC}, the most characteristic capacitative current. By analogy with voltage–operated calcium channels, if mammalian TRP proteins form calcium channels, it would require four units to form a channel. It is possible that physiologically these channels are heteromeric and that these heteromeric complexes behave differently compared to the homomers obtained when the protein is overexpressed in experimental systems. There is evidence that TRP1 and TRP3 associate when expressed

Table 1. Conductance behaviour of vertebrate TRP homologues.

TRP homologue	Species	Variants	Functional characterization
TRP1	Human	htrp1	Functions as SOC
		htrpC1	Nonselective cation channel
		htrpC1A	Calcium permeable cation channel; Functions as an SOC
	Mouse	mTRP1	Functional expression not yet reported
	Rat	rTRP1, rTRP1*b*	Functional expression not yet reported
	Cattle	b-TRP1	Functional expression not yet reported
	Xenopus laevis	x-TRP	Functional expression not yet reported
TRP2	Human	hTRP2	Expression not yet reported
	Mouse	mTRP2	Evidence for SOC function
	Cattle	bTRP2	Functional expression not yet reported
TRP3	Human	hTRP3	Conflicting reports regarding its function. Some studies favour it to be an SOC while more recent results show it to be conducting non-capacitative calcium current
		hTRPC3	Non selective cation channel activated by diacylglycerol (DAG)
	Mouse	mTRP3	Functional expression not yet reported
	Rat	r-TRP3	Functions as an SOC
TRP4	Human	hTRP4	Functional expression not yet reported
	Mouse	mTRP4	Non-selective cation channel. Does not function as an SOC
	Rat	rTRP4 (rTRP-R)	Results in favour of an SOC function
	Cattle	bTRP4 (bCCE1)	Results in favour of an SOC function
TRP5	Mouse	mTRP5	Non selective calcium channel. Does not function as an SOC
	Rat	rTRP5	Functional expression not yet reported
	Rabbit	TRP-5	Calcium channel. Does not function as an SOC
TRP6	Human	hTRP6	Non selective cation channel. Activated by DAG
	Mouse	mTRP6	Functions as an agonist induced calcium channel. Does not function as an SOC
	Rat	rTRP6	Functional expression not yet reported
TRP7	Mouse	mTRP7	Calcium selective cation channel. Does not function as an SOC. Activated by agonist (DAG).

(Harteneck 2000; Barritt 1999; Liu *et al* 2000; Schaefer *et al* 2000; Phillipp *et al* 2000).

together (Gillo *et al* 1996). Other possible explanations for the contradictory results include the use of different clones from different species and the use of different expression systems with, for example, the possibility of different endogenous TRP subunit combinations. The only general conclusion that can be drawn from the results present so far is that TRP homologues form functionally important calcium channels, with some of them being activated by internal store depletion. Continued work in this field will eventually disclose further properties which will help in fully understanding the functions of these interesting proteins and their relation to the genuine SOC.

3. Activation of the SOCs: the signalling mechanism behind it

Another major unresolved problem in the field of capacitative calcium entry is the mechanism of activation of the SOCs. The key question is how a decrease in the concentration of calcium in the lumen, sensed by the SOC. Three main hypotheses have been proposed: (i) a diffusible message, (ii) conformational coupling and (iii) vesicle secretion (schematic in figure 2).

The diffusible messenger hypothesis proposes that a messenger molecule is generated that activates calcium entry in response to store depletion. Patch clamp studies in *Xenopus* oocytes provided a very strong support for this hypothesis (Parekh *et al* 1993). Numerous molecules have been reported to be acting as the diffusible messenger. These include small G proteins, ATP, GTP, pertussis toxin-sensitive heterotrimeric G proteins, a product of cytochrome P450 activity, arachidonic acid (for references for each see the review by Putney and McKay 1999), cGMP (Kwan *et al* 2000) but to date none has been demonstrated very convincingly. Perhaps the most interesting and controversial candidate for second messenger is calcium influx factor (CIF). CIF was initially isolated from Jurkat T cells stimulated to deplete their calcium stores (Randriamampita and Tsien 1993). It has been partially

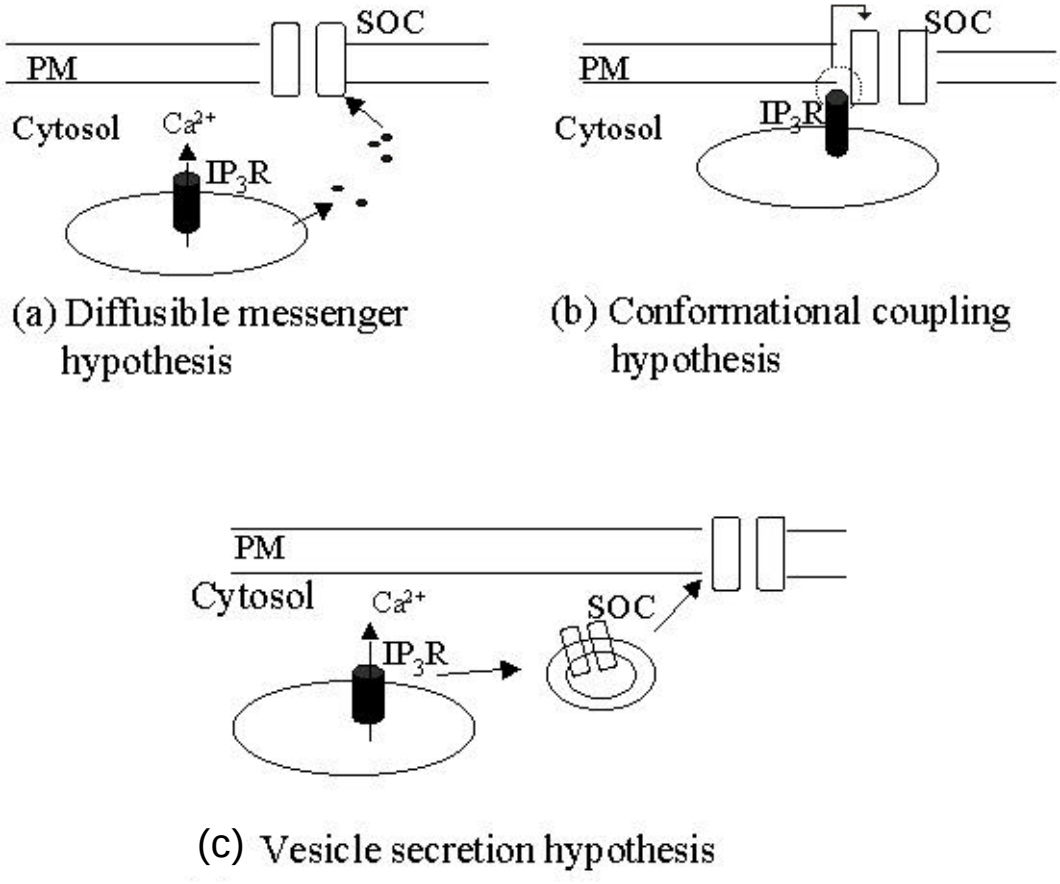

Figure 2. The three main hypotheses proposed for activation of SOC. **(a)** Diffusible messenger hypothesis: calcium store depletion generates a diffusible molecule (shown in black circles) which activates the SOCs in the plasma membrane **(b)** Confirmational coupling hypothesis: store depletion is conveyed to the SOCs by the direct physical binding of the IP_3R to the SOC (the physical interaction between the two molecules is shown by a dotted circle and the arrow indicates activation of the SOC) **(c)** Vesicle secretion hypothesis: store depletion leads to fusion of preformed vesicles, containing SOCs, to the plasma membrane.

characterized as a < 500 Da phosphorylated pH-stable anion that is presumably released or generated from the ER or adjacent regions after IP_3 induces calcium release from stores (Clapham 1993). After these initial studies, further results in favour of CIF came only as late as in 1999 from Richard Marchase's lab where they provided evidences for the existence of diffusible CIF in yeast cells and in mammalian smooth muscle cells (Csutora *et al* 1999; Trepakova *et al* 2000). However the exact nature of CIF yet remains unidentified. Clearly, the identification of CIF is the most important prerequisite for this model to gain acceptance.

The conformational coupling hypothesis postulates a direct interaction between the IP_3R in the ER membrane with the SOC in the plasma membrane. According to this model, the IP_3R senses the calcium depletion within the store and transduces this signal to SOC by physically coupling with it. The proposed mechanism is very similar to the known signalling interaction between the ryanodine receptor, a functional relative of IP_3R localized in the sarcoplasmic reticulum, and the calcium channel dihydropyridine receptor (DHPR), present on the plasma membrane of skeletal muscles. The conformational coupling hypothesis was predicted in the early nineties (Berridge 1995) but could not be convincingly proved at that time. With the TRP homologues proposed to be constituting the SOCs, they became the focus of further investigation. Co-immunoprecipitation studies showed direct physical interaction between *Drosophila* TRP and IP_3R (Boulay *et al* 1999). Studies done in the cell culture system (Kiselyov *et al* 1998; 1999; Hong Tao *et al* 2000) showed that IP_3 receptor interact with human TRP3 and activate it. A study by Kiselyov *et al* (1999) indicated that the N terminal region of the IP_3R is involved in TRP activation. These results however are not without drawbacks. The cell culture studies have used the human TRP3 as a model protein for SOC function. But the role of hTRP3 as a SOC itself is ambiguous (see table 1). More importantly, the result showing co-immunoprecipitation between *Drosophila* TRP and IP_3R might have little significance with the recent findings showing TRP activation to be independent of IP_3R function (Raghu *et al* 2000). Furthermore, there have also been studies in the recent past (Jayaraman *et al* 1995; Sugawara *et al* 1997) wherein, using IP_3R mutated cell lines or IP_3R antisense RNAs, researchers have ruled out the role of IP_3R in capacitative calcium entry. Another approach, in support of the conformational coupling hypothesis has been to focus on the spatial localization of the IP_3R with respect to the SOCs. For this model to work, these two multimeric proteins must be sufficiently close so that they can interact with each other. Evidence favouring such close association has come from cell fractionation studies which reveal that IP_3R co-purifies with plasma membrane fractions (Lievremont *et al* 1994). Furthermore, it has been observed that site of capacitative current entry is in close proximity to the site of IP_3 induced calcium release, leading to the speculation that SOC and IP_3R are closely apposed and therefore could interact physically (Peterson *et al* 1996; Jaconi *et al* 1997). However, given the close proximity of the IP_3R with the SOCs, one would expect rapid activation of SOCs but in reality the activation of SOCs is rather slow (10–100 s) (Kerschbaum and Cahalan 1999).

The third model for explaining SOC is the vesicle-mediated channel insertion hypothesis in which it is proposed that the decrease in store calcium causes additional preformed SOC proteins to be inserted in the plasma membrane. Apart from a few pharmocological studies, there were not enough results to support the secretion-like mechanism. Vesicle trafficking inhibitors like primaquine were found to affect thapsigargin-activated calcium entry, with no effect on the IP_3 induced calcium mobilization (Somasundaram *et al* 1995). Two recent papers provide fresh evidence in support of this mechanism (Patterson *et al* 1999; Yao *et al* 1999). In one of the papers (Yao *et al* 1999), Yao and coworkers have shown SNAP-25, one of the proteins necessary for membrane fusion to be involved in SOC function. Involvement of SNAP-25 adds a new twist to the tale and opens up many plausible hypotheses and experiments that can be tested.

The mechanism of activation of the SOCs still remains unclear, with most of the recent findings favouring either the coupling model or the secretion model. Possibly, the actual mechanism of activation is in between these two models. In figure 3 are shown two proposed models, both of which try to take into account the data of the secretion as well as the coupling model of activation. In model A, the SOCs are present in semidocked vesicles and are associated loosely with the IP_3R. A depletion in calcium signal results in a stronger interaction between the two proteins which results in complete fusion of these semi docked vesicles with the plasma membrane (a process dependent on SNAP25) and the consequent activation of the SOC. In model B, store depletion first results in the docking of the ER containing IP_3R near the cell surface (which could explain the longer time required for capacitative current entry) succeeded by the physical binding of the IP_3R with the SOCs and activating them. Both of these models support the idea of a close, intimate interaction between the ER calcium stores and PM channel. However, the role of the diffusible messengers cannot be entirely ruled out and the possibility of this mechanism existing in a few cell types always remains.

4. Future research

There are obviously various issues that need to be resolved. The channel protein having conductance properties like

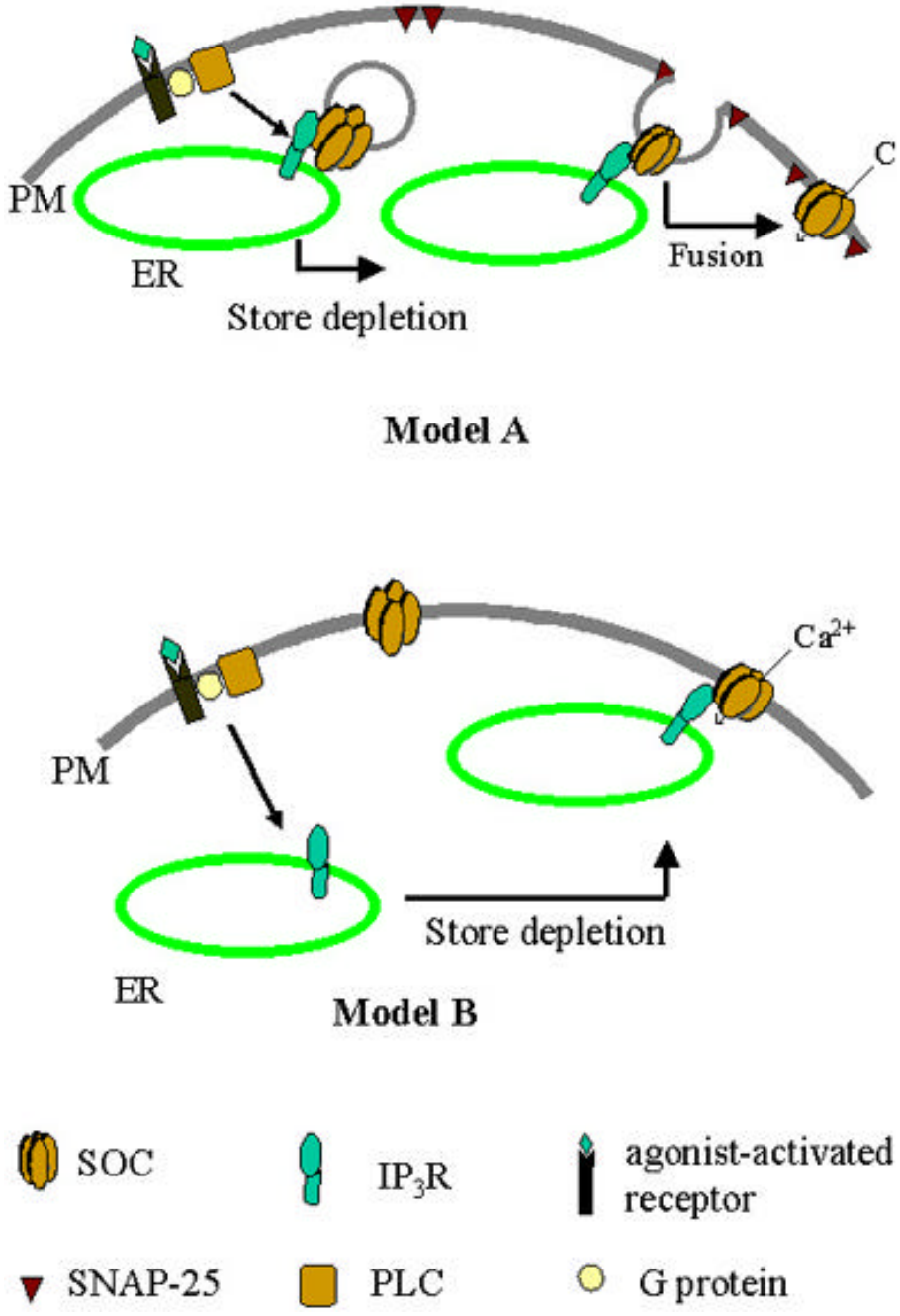

Figure 3. Proposed models for SOC activation (based on recent data). Upon receiving appropriate stimulation, the G-protein coupled receptor activates phospholipase C (PLC). Activated PLC generates IP_3, depleting intracellular calcium stores in the endoplasmic reticulum (ER). *Model A*: The SOCs are present in vesicles within the cytosol. In the resting stage (when the calcium stores are full), the IP_3Rs are weakly bound to the SOCs. Upon store depletion, the coupling between IP_3R and SOCs is tightened, possibly by changes occurring in protein/s conformation. This leads to the complete fusion of the SOC vesicles to the plasma membrane (PM), activation of the SOC and subsequent entry of calcium in the cell. SNAP-25, which is a t-SNARE, is present in the PM and mediates the fusion of SOC vesicles with the PM. *Model B*: At the resting stage, the SOCs are present in the PM in an inactivated state. There exists a gap between the calcium stores in the ER region and the PM. Calcium store depletion first results in the movement of the calcium stores close to the PM. This is succeeded by the physical coupling of the IP_3R with the SOC resulting in the activation of the SOC and entry of capacitative current. This model does not include any membrane fusion and hence, does not take into account the role of SNAP-25.

In this schematic, the SOCs have been hypothesized to be present as multimers. Also, the inactivated SOC (either in weak association with the IP_3R in model A or in the PM in model B) has been shown in a slightly different conformation than its activated state. The sizes of the various molecules and vesicles are not according to scale.

I_{CRAC} is still not identified and the hunt for such a protein is on. The nature of the endogenous calcium channel is not yet known – is it a homomer, heteromer or a complex comprising of yet unknown protein molecules? Even the various functional domains of TRP proteins, the channel forming domain and the IP_3R interacting domain, needs to be dissected carefully. In spite of a lot of efforts, the knowledge regarding the mechanism of activation of SOC is still fragmentary. Further work in these directions will help unravel the fascinating phenomenon of capacitative calcium entry.

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