REVIEW ARTICLE

Receptor-activated Ca^{2+} inflow in animal cells: a variety of pathways tailored to meet different intracellular Ca^{2+} signalling requirements

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Receptor-activated Ca^{2+} channels (RACCs) play a central role in regulation of the functions of animal cells. Together with voltage-operated Ca^{2+} channels (VOCCs) and ligand-gated non-selective cation channels, RACCs provide a variety of pathways by which Ca^{2+} can be delivered to the cytoplasmic space and the endoplasmic reticulum (ER) in order to initiate or maintain specific types of intracellular Ca^{2+} signal. Store-operated Ca^{2+} channels (SOCs), which are activated by a decrease in Ca^{2+} in the ER, are a major subfamily of RACCs. A careful analysis of the available data is required in order to discern the different types of RACCs (differentiated chiefly on the basis of ion selectivity and mechanism of activation) and to properly develop hypotheses for structures and mechanisms of activation. Despite much intensive research, the structures and mechanisms of activation of RACCs are only now beginning to be understood. In considering the physiological functions of the different RACCs, it is useful to consider the specificity for Ca^{2+} of each type of cation channel and the rate at which Ca^{2+} flows through a single open channel; the locations of the channels on the plasma membrane (in relation to the ER, cytoskeleton and other intracellular units of structure and function); the Ca^{2+}-responsive enzymes and proteins; and the intracellular buffers and proteins that control the distribution of Ca^{2+} in the cytoplasmic space. RACCs which are non-selective cation channels can deliver Ca^{2+} directly to specific regions of the cytoplasmic space, and can also admit Na^+, which induces depolarization of the plasma membrane, the opening of VOCCs and the subsequent inflow of Ca^{2+}. SOCs appear to deliver Ca^{2+} specifically to the ER, thereby maintaining oscillating Ca^{2+} signals.

Key words: endoplasmic reticulum, inositol 1,4,5-trisphosphate, non-selective cation channels, store-operated Ca^{2+} channels, transient-receptor-potential homologues.

INTRODUCTION

Changes in the cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_c$) constitute one of the main pathways by which information is transferred from extracellular signals received by animal cells to intracellular sites [1–3]. The intracellular Ca^{2+} signal is conveyed by the magnitude, location and duration of the changes in $[Ca^{2+}]_c$ [4–6]. Increases in $[Ca^{2+}]_c$ in a given region of the cytoplasmic space are usually initiated by the binding of an extracellular signalling molecule (agonist) to its plasma-membrane receptors [1–6].

The mechanisms by which the increase in $[Ca^{2+}]_c$ is achieved differ somewhat between 'excitable' cells, which exhibit an action potential or a substantial general depolarization of the plasma membrane in response to a depolarizing stimulus [7], and 'non-excitable' cells [6]. In excitable cells, increases in $[Ca^{2+}]_c$ result from the inflow of Ca^{2+} through plasma-membrane Ca^{2+} channels and/or the release of Ca^{2+} from the sarcoplasmic reticulum through ryanodine-receptor Ca^{2+} channels [8–10]. In non-excitable cells, the increase in $[Ca^{2+}]_c$ results from the inflow of Ca^{2+} across the plasma membrane and the release of Ca^{2+} from the endoplasmic reticulum (ER). The latter is induced by inositol 1,4,5-trisphosphate ($Ins P_3$), which binds to $Ins P_3$ receptor Ca^{2+} channels, and by Ca^{2+}, which binds to ryanodine-receptor Ca^{2+} channels [1–3,6]. Many other processes influence the magnitude, location and duration of agonist-induced increases in $[Ca^{2+}]_c$. These include the sarcoplasmic and endoplasmic reticulum ($Ca^{2+}+Mg^{2+}$)-ATPases (SERCAs), which transport Ca^{2+} into these organelles, plasma-membrane ($Ca^{2+}+Mg^{2+}$)-ATPases (PMCAs), which transport Ca^{2+} out of the cells across the plasma membrane, the mitochondrial Ca^{2+} transporters and channels, and the cytoplasmic and intra-organelle proteins and metabolites that bind Ca^{2+} [1–3,6].

There are three main pathways by which extracellular Ca^{2+} can enter animal cells. These are voltage-operated Ca^{2+} channels (VOCCs), ligand-gated non-specific cation channels (LGCCs) and receptor-activated Ca^{2+} channels (RACCs) [1–3,6,9–11]. The broad physiological functions of these channels are to provide extracellular Ca^{2+} for increases in $[Ca^{2+}]_c$ in given regions of the cytoplasmic space, and to replenish Ca^{2+} stores in the sarcoplasmic reticulum and the ER by replacing Ca^{2+} which is released from these stores and subsequently pumped out of the cell [1–3,6,9–11]. In so far as it can be ascertained from electrophysiological studies, the members of all of these families of plasma-membrane channels exhibit the electrophysiological properties usually attributed to a cation channel [7]. That is, when induced to open, the channel remains open for a short period of time (usually microseconds) before closing as the result of an inactivation mechanism [7]. Activation of a channel involves an increase in the probability of channel opening [7]. During the

Abbreviations used: RACC, receptor-activated Ca^{2+} channel; VOCC, voltage-operated Ca^{2+} channel; LGCC, ligand-gated Ca^{2+} channel; SOC, store-operated Ca^{2+} channel; CRAC, Ca^{2+}-release-activated Ca^{2+} channel; I_{CRAC}, current carried by ions moving through CRACs; ER, endoplasmic reticulum; SERCA, sarcoplasmic/endoplasmic reticulum ($Ca^{2+}+Mg^{2+}$)-ATPase; PMCA, plasma-membrane ($Ca^{2+}+Mg^{2+}$)-ATPase; $[Ca^{2+}]_c$, cytoplasmic free Ca^{2+} concentration; $[Ca^{2+}]_{er}$, concentration of Ca^{2+} in the lumen of the ER; $[Ca^{2+}]_o$, extracellular Ca^{2+} concentration; $[Na^+]_c$, cytoplasmic free Na^+ concentration; $Ins P_3$, inositol 1,4,5-trisphosphate; $Ins P_4$, inositol 1,3,4,5-tetrakisphosphate; GTP[S], guanosine 5′-[γ-thio]triphosphate; TRP, transient receptor potential; TRPL, transient receptor potential-like; CHO, Chinese-hamster ovary; RBL, rat basal leukaemia.

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open period, the inflow of Ca^{2+} or Na^+ through the channel can be described by the Goldman–Hodgkin–Katz equation [7]. The driving force for Ca^{2+} entry is the magnitude of the membrane potential and the magnitude of the Ca^{2+} gradient across the plasma membrane [7].

Of all the plasma-membrane Ca^{2+} channels, the RACCs are the most poorly understood [1–3,6,12–15]. This incomplete knowledge may be partly because there are a large number of RACC subtypes. Thus a given type of animal cell possesses several types of RACC, each most probably having a different physiological function, and different electrophysiological properties, structure and mechanism of activation [11,12]. Moreover, it is likely that cells with a variety of different functions require different forms of RACC. The ways in which each of these different types of RACC contributes to specific patterns of increase in $[Ca^{2+}]_c$ are presently not well understood. Investigation of the structures and functions of RACCs may have also been made more difficult by a tendency of researchers not to keep experimental observations of the nature and properties of RACCs separate from the initial stages of the development of hypotheses for the structures and mechanisms of channel opening.

The aim of this review is to summarize current knowledge of the molecular nature and physiological functions of RACCs. This will be done by considering the experimental data which are beginning to allow the different types of RACCs to be identified more clearly; by summarizing current knowledge of the structures and mechanisms of activation of the RACCs; and by formulating questions about (i) the ways in which the opening of the different types of RACCs leads to increases in the Ca^{2+} concentration in specific regions of the cell, and (ii) how different types of RACC may have evolved to fulfil specific intracellular signalling functions. An underlying theme is that the different types of RACCs have quite specific, but not yet well understood, roles in delivering a given type of Ca^{2+} signal to the cell.

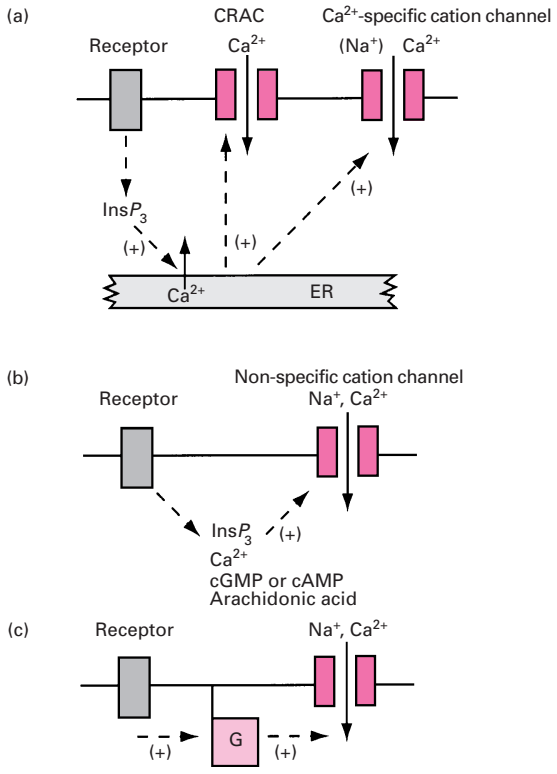

Figure 1 Types of RACC

The two main types of RACC in animal cells are (**a**) SOCs and (**b**) intracellular-messenger-activated non-selective cation channels. A third possibility (**c**) is non-selective cation channels activated by a trimeric G-protein (G).

DIFFERENT TYPES OF RACCs IN ANIMAL CELLS

Definition of RACCs

A RACC is defined here as any plasma-membrane Ca^{2+} channel, other than a VOCC, which is opened as a result of the binding of an agonist to its receptor, where the receptor protein is separate from the channel protein and for which the mechanism of channel opening does not involve depolarization of the plasma membrane [1–3,11,12]. This definition implies some mechanism, such as a mobile intracellular messenger, a trimeric G-protein, or the involvement of the ER, to link the receptor protein to the Ca^{2+}-channel protein [1–3,11,12].

There are numerous subtypes of RACCs. These can be differentiated on the basis of their selectivity for cations, mechanisms of channel opening and physiological functions [2,11,12] (Figure 1). The Ca^{2+}-selectivity of RACCs varies from those that are quite selective for Ca^{2+}, called here Ca^{2+} channels, and those that are much less selective for Ca^{2+}, called here non-selective cation channels [2,11,12]. Under physiological conditions, members of the last-mentioned group most probably admit predominantly Na^+, but also admit sufficient Ca^{2+} to cause a significant increase in $[Ca^{2+}]_c$.

The subgroup of RACCs that has recently received most attention is the store-operated Ca^{2+} channels (SOCs) (capacitative Ca^{2+} channels) [1–3,12–18] (Figure 1a). These are defined as plasma-membrane Ca^{2+} channels that are opened in response to a decrease in the concentration of Ca^{2+} in the lumen of the ER ($[Ca^{2+}]_{er}$) [1]. Under physiological conditions the decrease in $[Ca^{2+}]_{er}$ that leads to channel opening is caused by the binding of $InsP_3$ to $InsP_3$-receptor Ca^{2+} channels [1,2,12], and possibly also by the binding of Ca^{2+} to ryanodine-receptor Ca^{2+} channels [19,20]. The key event that initiates the opening of SOCs is the decrease in $[Ca^{2+}]_{er}$ [1,2,12]. As discussed below, the mechanism(s) by which this event induces the opening of these plasma-membrane Ca^{2+} channels has not been unequivocally defined.

Agonists can induce the opening of more than one type of RACC in many cell types

In many types of cells, agonists can open more than one type of RACC. For example, in portal-vein smooth-muscle cells the binding of noradrenaline to α_{1A}-adrenergic receptors leads to the opening of both a SOC subtype and a subtype of non-selective cation channel [21]. Activation of the latter is thought to involve the combined actions of an increase in $[Ca^{2+}]_c$ and a trimeric G-protein [21] (Figure 2). In addition to these two types of RACCs, noradrenaline and angiotensin II can open an L-type VOCC in smooth-muscle cells, and these cells also possess T-type VOCCs [21]. Multiple plasma-membrane Ca^{2+} channels have also been described in cultured smooth-muscle cells [22] and in human embryonic kidney cells [23]. In the A7r5 smooth-muscle cell line (derived from rat aorta), vasopressin stimulates Ca^{2+} inflow through SOCs and through one other RACC subtype [22].

In mast cells, electrophysiological experiments have clearly shown the presence of one subtype of SOC and another RACC subtype which is a non-selective cation channel thought to be opened by the action of a trimeric G-protein [12,24]. A third example of a cell expressing multiple RACCs is the hepatocyte. In rat hepatocytes, at least two forms of RACCs with different

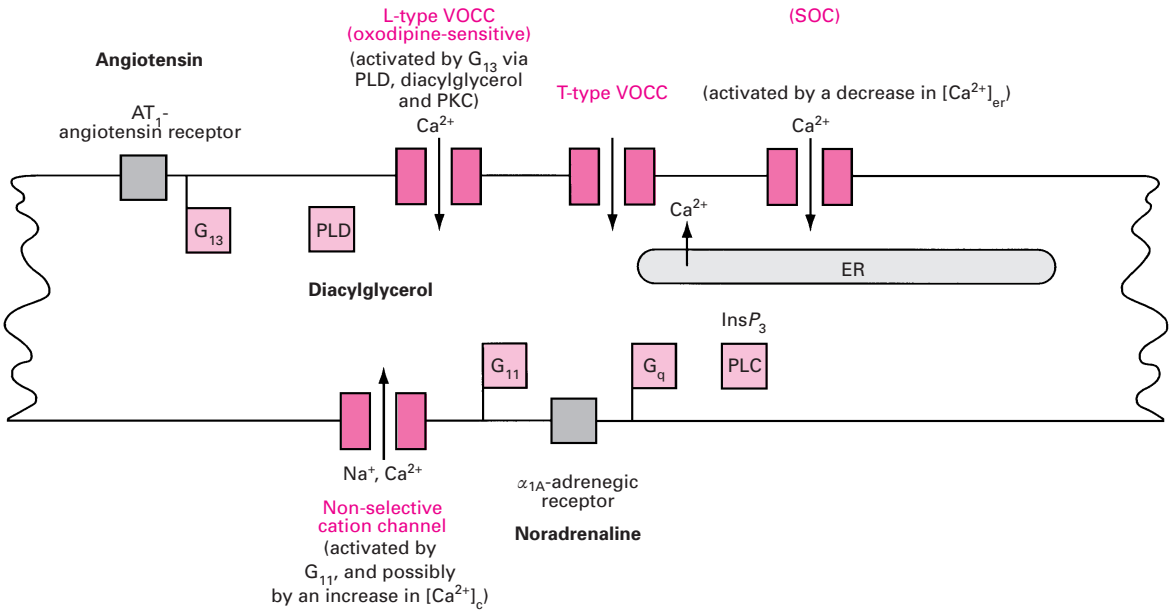

Figure 2 Most animal cells possess several different types of plasma-membrane Ca^{2+} channels, which allow the generation of different types of intracellular Ca^{2+} signals

The Figure shows a representation of the main types of plasma-membrane Ca^{2+} channels that have been shown to be present, or which are proposed to be present, in rat portal-vein myocytes, and summarizes the results and interpretation derived from recent work by Macrez-Lepêtre et al. [21]. While some of the pathways of channel activation are yet to be confirmed, the Figure provides a good illustration of the complexity of plasma-membrane Ca^{2+} channels in one animal cell. Noradrenaline is proposed to activate: (1) SOCs {via G_q, phospholipase Cβ (PLC), $InsP_3$ and a decrease in $[Ca^{2+}]_{er}$}; (2) non-specific cation channels (via G_{11} and possibly $[Ca^{2+}]_c$) and (3) L-type and T-type VOCCs (via depolarization of the plasma membrane induced by opening of non-selective cation channels). Angiotensin II is proposed to activate L-type VOCCs [via G_{13}, phospholipase D (PLD), diacylglycerol and protein kinase C (PKC)].

apparent selectivities for Ca^{2+} are opened in response to thapsigargin [25,26]. Rat hepatocytes may possess a third type of RACC that is opened in response to an increase in the concentration of cytoplasmic cAMP (reviewed in [27]). Direct evidence for a cAMP-activated channel in hepatocytes comes from studies of axolotl hepatocytes, which possess plasma-membrane Ca^{2+} channels that are opened by an increase in cAMP [28]. Further evidence for the presence of multiple subtypes of RACCs in hepatocytes comes from studies of liver tumour cell lines which express non-selective cation channels that are opened in response to an increase in $[Ca^{2+}]_c$ [29].

EXPERIMENTAL OBSERVATIONS THAT DEFINE THE DIFFERENT TYPES OF RACC

Channels activated by mobile intracellular messengers

There is evidence that non-excitable cells express RACCs that are opened by the action of cGMP [30], cAMP [28,30], $InsP_3$ [31–36] [and possibly inositol 1,3,4,5-tetrakisphosphate ($InsP_4$)] [37]) and arachidonic acid (or an arachidonic acid metabolite) [23,38–40] (Figure 1b). The most clearly described Ca^{2+} channels that are opened by the action of an intracellular messenger are the cGMP-activated non-selective cation channels present in mammalian retinal cells (reviewed in [30]). There is also good evidence for the existence of non-selective cation channels that are activated by cyclic nucleotides in olfactory and gustatory cells [30].

The idea that there are plasma-membrane Ca^{2+} channels that are activated by $InsP_3$ is based on the observation that $InsP_3$ stimulates Ca^{2+} inflow in isolated membrane patches [31–35] and on evidence suggesting that some $InsP_3$ receptor proteins are located on the plasma membrane [36]. There is also evidence which suggests that $InsP_3$ activates a non-selective cation channel in rat megakaryocytes [41], although under physiological conditions this channel may chiefly admit Na^+.

In interpreting the results of these experiments, which imply the presence of $InsP_3$-receptor Ca^{2+} channels in the plasma membrane, it has proven very difficult to differentiate between $InsP_3$ receptor Ca^{2+}-channel proteins located in the ER close to, and/or attached to, the plasma membrane (which could activate SOCs in the plasma membrane) and $InsP_3$-activated Ca^{2+} channels located in the plasma membrane itself [35,36,42]. Comparison of the electrophysiological properties of $InsP_3$ receptor Ca^{2+} channels introduced into synthetic bilayers [43] or of $InsP_3$ receptor Ca^{2+} channels *in situ* in the nuclear membrane [44] with the electrophysiological properties of known RACCs suggests that, if any of the known $InsP_3$ receptor Ca^{2+}-channel subtypes are located in the plasma membrane, they would exhibit a reasonably high cation conductance. The results of experiments (described below) conducted with the *Drosophila melanogaster* TRPL [transient receptor potential (TRP)-like] non-selective cation channel either *in situ* in *Drosophila* photoreceptor cells (reviewed in [13–15]) or expressed heterologously in insect or animal cells [45,46] suggest that this non-specific cation channel is activated by $InsP_3$. Thus it can be concluded that, while it is possible that some animal cell RACC proteins are $InsP_3$ receptor Ca^{2+} channels located in the plasma membrane, further experiments are required to test this idea.

Results which suggest that there are $InsP_4$-activated Ca^{2+} channels in the plasma membrane are also difficult to interpret [12]. At present there is no convincing evidence to indicate that $InsP_4$ directly activates any plasma-membrane Ca^{2+} channels. However, it is possible that some of the observed effects of $InsP_4$ on Ca^{2+} inflow may be due to the interaction of $InsP_4$ either with

an InsP_3 receptor Ca^{2+}-channel protein or an InsP_4-binding protein located in the ER near the plasma membrane, or with an InsP_4-binding protein in the plasma membrane. Subsequent interaction of the InsP_3 receptor Ca^{2+}-channel protein or the InsP_4-binding protein with the RACC protein in the plasma membrane may lead to the opening of a plasma-membrane Ca^{2+} channel [47].

Evidence for a role for arachidonic acid, or an arachidonic acid metabolite, in inducing the opening of plasma-membrane Ca^{2+} channels has been obtained in a variety of cell types. In human A431 carcinoma cells and in murine P19 embryonic carcinoma cells transfected with epidermal growth factor receptors, the binding of epidermal growth factor to its receptor has been shown to induce the generation of the arachidonic acid metabolite leukotriene C_4, which activates a Ca^{2+} channel with a conductance of approx. 10 pS [38]. Shuttleworth [23,39] has shown that arachidonic acid itself, formed in response to the interaction of carbachol with a muscarinic acetylcholine receptor, activates plasma-membrane Ca^{2+} channels in exocrine avian nasal gland cells and in human embryonic kidney cells. Moreover, evidence has been presented to indicate that the activation of Ca^{2+} inflow into Balb-C 3T3 fibroblasts by basic fibroblast growth factor is mediated by arachidonic acid [40]. Taken together, these results suggest that RACCs opened by the action of arachidonic acid itself or by an arachidonic acid metabolite are present in the plasma membranes of a number of cell types. It has also been suggested, as discussed below (see Table 2), that arachidonic acid may mediate the activation of some SOCs [48].

Channels activated by intracellular Ca^{2+}

Evidence for the presence of Ca^{2+}-activated non-selective cation channels which could be candidates for Ca^{2+}-activated RACCs has been obtained in studies of a number of cell types [29,49–54]. In human neutrophils [53] and liver tumour cell lines [29], these channels have been characterized using electrophysiological techniques. As discussed below, some Ca^{2+}-activated Ca^{2+} channels may be opened by Ca^{2+} released from the ER in response to an agonist-induced increase in the InsP_3 concentration.

Some plasma-membrane Ca^{2+} channels may be activated by direct interaction between the channel protein and a trimeric G-protein

The possibility that the opening of some types of RACC can be induced by the direct interaction of the channel protein with either the α subunit or the $\beta\gamma$ subunit of a trimeric G-protein (released following the binding of an agonist to its receptor), in a process limited to the plane of the plasma membrane (Figure 1c), has been considered for some time [11,12,55–58]. However, as in the case of the activation of K^+ channels and VOCCs by trimeric G-proteins [59], it has been difficult to devise definitive tests of this putative activation mechanism. Evidence for the idea that G-proteins may activate RACCs directly has come from a variety of experimental approaches.

Studies with mast cells [55], smooth-muscle cells [21,57] and HL-60 cells (a neutrophil cell line) [58] using patch-clamp electrophysiological techniques have shown that guanosine 5′-[γ-thio]triphosphate (GTP[S]), an activator of trimeric G-proteins, activates non-selective cation channels which are most probably a RACC subtype. The argument for the direct activation of RACCs by a trimeric G-protein is the observation that, in electrophysiological experiments employing the inside-out patch-clamp recording configuration, GTP[S], when present on the cytoplasmic side of the membrane (i.e. in the bath solution), activates plasma-membrane Ca^{2+} channels. Under these experimental conditions, any mobile intracellular messenger that might be formed as a result of the action of GTP[S] would most probably be immediately diluted by rapid diffusion into the surrounding medium [58]. While these observations do provide some evidence for a direct G-protein–RACC interaction, they do not exclude the involvement of a mobile messenger [58]. Other experiments (e.g. the use of soluble G-protein subunits or reconstitution experiments) are required to further test the idea of a direct G-protein–RACC protein interaction.

Experiments with portal-vein smooth-muscle cells have shown that, in addition to opening SOCs, noradrenaline induces the opening of non-selective cation channels by a mechanism that does not appear to involve Ca^{2+} release from the ER [21]. Anti-G-protein antibodies and antisense DNA techniques have been used to establish a requirement for $G_{11}\alpha$ in the mechanism by which noradrenaline induces the opening of these non-selective cation channels [21]. Thus it is proposed that noradrenaline-induced increases in $[Ca^{2+}]_c$ in portal-vein smooth-muscle cells involve (1) the generation of InsP_3 (via G_q and phospholipase C), which leads to the release of Ca^{2+} from the ER and to the opening of SOCs, and (2) the opening of non-selective cation channels via G_{11} and a presently unknown link between G_{11} and the non-selective cation channel [21] (Figure 2).

The pathway by which the binding of carbachol to M_3-muscarinic receptors, expressed in CHO (Chinese hamster ovary) cells or A9 fibroblasts, leads to an increase in $[Ca^{2+}]_c$ also provides evidence which suggests that a RACC in these cells is activated by direct interaction with a trimeric G-protein [60,61]. Thus it has been shown that the third cytoplasmic loop of the M_3-muscarinic receptor, which is known to be required for the activation of trimeric G-protein α subunits, is required for the activation of Ca^{2+} inflow, but not for the release of Ca^{2+} from the ER [60,61]. Another piece of indirect evidence which indicates that some animal cell RACCs may be directly activated by a trimeric G-protein comes from experiments which suggest that the *Drosophila* TRPL non-specific cation channel (defined below) expressed in insect Sf9 cells is opened in response to a direct interaction between $G_{11}\alpha$ and the TRPL protein [62].

While the results of all these experiments can be interpreted as indicating that some RACCs can be opened by direct interactions between a trimeric G-protein and the RACC protein, other explanations, such as the generation of a mobile intracellular messenger in response to activation of the G-protein, are also plausible. It is also interesting to note that, in addition to a proposed role for trimeric G-proteins in the membrane-delimited activation of RACCs, trimeric and monomeric G-proteins are required in the process by which the decrease in $[Ca^{2+}]_{er}$ leads to the opening of SOCs [56,63–66]. As discussed below, these G-proteins may play a permissive role in the process by which SOCs are opened, and are considered unlikely to interact directly with SOC proteins.

Channels activated by the release of Ca^{2+} from the ER (SOCs)

Initial evidence for store-operated ('capacitative') Ca^{2+} inflow

Since the idea of store-operated Ca^{2+} inflow (Figure 1a) was first proposed [67,68], it has become evident that there are several types of SOCs, which can be differentiated on the basis of their selectivity for Ca^{2+} compared with univalent cations (reviewed in [12]). Moreover, in the absence of electrophysiological data, there are considerable experimental difficulties in deciding whether a given plasma-membrane Ca^{2+} channel is actually opened in response to a decrease in $[Ca^{2+}]_{er}$. In order to appreciate

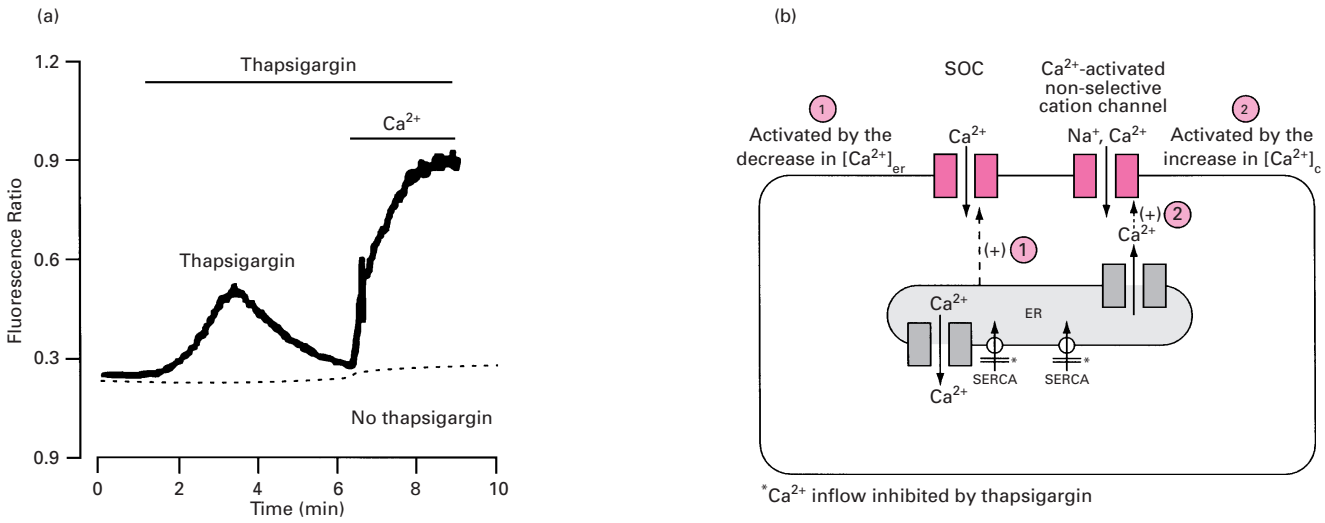

Figure 3 Interpretation of results obtained using thapsigargin and an intracellular fluorescent Ca^{2+} chelator to test for the presence of SOCs

(**a**) An example of the $Ca^{2+}{}_o$-add-back protocol, which is often used in conjunction with the treatment of cells with thapsigargin. The results shown were obtained for rat hepatocytes using fura-2 (microinjected into the cytoplasmic space) to measure changes in $[Ca^{2+}]_c$ [149]. The resulting increase in $[Ca^{2+}]_c$ compared with the control (no thapsigargin pretreatment) is due to increased Ca^{2+} inflow through SOCs and the inhibition by thapsigargin of Ca^{2+} inflow to the ER. (**b**) Representation of two mechanisms by which thapsigargin pretreatment can lead to an increase in $[Ca^{2+}]_c$ in the $Ca^{2+}{}_o$-add-back experimental protocol shown in (**a**).

these issues, it is useful to briefly review the types of experiments that led to the identification of SOCs.

In 1982, J. Putney and colleagues observed that, when lacrimal acinar cells were treated with acetylcholine in the absence of added extracellular Ca^{2+} ($Ca^{2+}{}_o$), in order to release Ca^{2+} from intracellular stores (most likely the ER), then treated with an acetylcholine antagonist to block the action of acetylcholine, a substantial inflow of Ca^{2+} was observed when $Ca^{2+}{}_o$ was then added [1,68,69]. This Ca^{2+} inflow, which was indistinguishable from, and not additive to, that induced by the agonist, depended on the prior release of Ca^{2+} from the ER and took place when there was no downstream signal emanating from the acetylcholine receptor (blocked by the antagonist) [1,68,69]. Thus it was concluded that a decrease in $[Ca^{2+}]_{er}$ could initiate activation of plasma-membrane Ca^{2+} inflow [1,68,69].

Two further discoveries provided additional evidence for the phenomenon of store-activated Ca^{2+} inflow. Firstly, thapsigargin, a SERCA inhibitor, was discovered (reviewed in [1]). Treatment of cells with thapsigargin, or another SERCA inhibitor (2,5-di-t-butylhydroquinone or cyclopiazonic acid), in the absence of added Ca^{2+} was found to mimic natural agonists by releasing Ca^{2+} from the ER and stimulating Ca^{2+} inflow when the extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) was increased (in the absence of agonist–receptor interaction) (Figure 3a) (reviewed in [1]). Secondly, it was observed that in many, but not all, non-excitable cells, treatment with thapsigargin or other manipulations that decrease $[Ca^{2+}]_{er}$ stimulates Mn^{2+} inflow across the plasma membrane (reviewed in [1]). It was concluded that (1) some SOCs are permeable to Mn^{2+} as well as Ca^{2+}, and (2) this observation, essentially an alternative assay for plasma-membrane bivalent-cation inflow, provided additional evidence for the existence of SOCs (reviewed in [1]).

Experiments were conducted with a few cell types to show that the activation of Ca^{2+} or Mn^{2+} inflow across the plasma membrane induced by a decrease in $[Ca^{2+}]_{er}$ was unlikely to be due to the increase in $[Ca^{2+}]_c$ resulting from the release of Ca^{2+} from the ER and the subsequent activation of the plasma-membrane bivalent-cation channel by Ca^{2+} itself. Thus it was concluded that, for the cell types tested, SERCA-inhibitor-induced Ca^{2+} inflow was activated by the decrease in $[Ca^{2+}]_{er}$ (reviewed in [1]).

Ca^{2+}-release-activated Ca^{2+} currents

Utilization of patch-clamp electrophysiological techniques provided a substantial advance in knowledge of the properties of SOCs. This technique allowed the identification in lymphocytes and mast cells of plasma-membrane Ca^{2+} channels that could be opened by manipulations that induce the release of Ca^{2+} from the ER (e.g. thapsigargin or another SERCA inhibitor; $InsP_3$ or a slowly-metabolizable $InsP_3$ analogue; or ionomycin) [12,16–18]. These channels were called Ca^{2+}-release-activated Ca^{2+} channels (CRACs), and the current [usually measured in the cell-attached (whole cell) mode] carried by ions moving through them was termed I_{CRAC} [12,16–18]. These experiments generally required dialysis of the cell with EGTA or BAPTA [bis-(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetra-acetic acid] in order to prevent feedback inhibition of CRACs by cytoplasmic Ca^{2+} (reviewed in [12]). CRACs have some distinctive characteristics, including a very low unitary conductance (about 20 fS), a high specificity for Ca^{2+}, the ability to conduct Ca^{2+} better than Ba^{2+} (a selectivity order of $Ca^{2+} > Ba^{2+} > Mn^{2+}$), a distinctive dependence of I_{CRAC} on $[Ca^{2+}]_o$, and an increase in the probability of channel opening at hyperpolarized membrane potentials (reviewed in [12,16–18]).

Difficulty in distinguishing experimentally between channels activated by a decrease in Ca^{2+} in the ER and those activated by an increase in Ca^{2+} in the cytoplasmic space

As described above, a general protocol used to test for the presence of SOCs in a given cell type utilizes thapsigargin or another SERCA inhibitor that is added to cells in the absence of added $Ca^{2+}{}_o$, followed by a $Ca^{2+}{}_o$ add-back procedure (Figure 3a). However, this protocol alone generally cannot distinguish between plasma-membrane Ca^{2+} channels activated by a decrease

in $[Ca^{2+}]_{er}$ on the one hand or by an increase in $[Ca^{2+}]_c$ on the other [12] (Figure 3b). Thus, when $Ca^{2+}{}_o$ is added to a cell previously treated with a SERCA inhibitor or an agonist that increases InsP_3, and the SERCA inhibitor or agonist has not been removed, the state of this cell is not the same as that of a cell in which $[Ca^{2+}]_{er}$ has been lowered, the InsP_3 concentration is at the basal value and no SERCA inhibitor is present. For cells in this last-mentioned state, when $Ca^{2+}{}_o$ is added, Ca^{2+} will be likely to flow through 'basal' Ca^{2+} channels or open SOCs, and then through a small region of the cytoplasmic space into the ER, with little increase in $[Ca^{2+}]_c$ (reviewed in [1,68,70]). However, in cells treated with a SERCA inhibitor or in which the concentration of InsP_3 remains elevated, the accumulation of Ca^{2+} in the ER is prevented. Therefore the $Ca^{2+}{}_o$-induced increase in $[Ca^{2+}]_c$ in these cells may be faster and more substantial than that in cells in which $[Ca^{2+}]_{er}$ is low, InsP_3 is at the basal value and SERCA inhibitors are absent [70]. The resulting increase in $[Ca^{2+}]_c$ could activate Ca^{2+}-activated plasma-membrane Ca^{2+} channels if these are present (Figure 3b).

Thus there may be instances where thapsigargin-stimulated Ca^{2+} inflow has been attributed to the opening of SOCs, but is, in fact, due to the opening of Ca^{2+}-activated plasma-membrane Ca^{2+} channels. Distinguishing between these possibilities (Figure 3b) is not easy [12]. The addition of Mn^{2+} in place of $Ca^{2+}{}_o$ to cells previously treated with an agent that lowers $[Ca^{2+}]_{er}$ offers one procedure which may distinguish between SOCs and Ca^{2+}-activated non-selective cation channels, although it is possible that with this protocol the latter are activated by the prior increase in $[Ca^{2+}]_c$ that occurs when Ca^{2+} is released from the ER [12]. The most definitive experiments for the identification of SOCs employ the patch-clamp technique together with an intracellular Ca^{2+} chelator to maintain a low $[Ca^{2+}]_c$ [12].

Different types of SOCs and store-operated non-selective cation channels in animal cells

Notwithstanding the problems just mentioned, it can be concluded that one or more subtype(s) of SOC are present in most types of non-excitable and in some excitable animal cells (reviewed in [1–3,12]). The majority of these SOCs have been detected by measuring changes in $[Ca^{2+}]_c$ in the presence of a SERCA inhibitor, as discussed above [1,2,12]. Channels with the characteristics of CRACs (the characteristic I_{CRAC}) have so far only been detected in lymphocytes and mast cells [12,16–18]. However, application of the patch-clamp technique to a number of other cell types has allowed the detection of other types of SOCs with characteristics similar to, but not identical with, those of CRACs. These cell types include macrophages, megakaryocytes, MDCK cells, 3T3 fibroblasts, hepatocytes, pancreatic acinar cells, endothelial cells (reviewed in [12,72]) and *Xenopus* oocytes [12,71]. Other putative SOCs, which are selective for bivalent cations but which exhibit a higher single-channel conductance (approx. 10 pS) and a different specificity for cations compared with that of CRACs, have been detected in endothelial cells and A431 cells (reviewed in [12]). Furthermore, there is evidence that in some cells a decrease in $[Ca^{2+}]_{er}$ can open non-selective cation channels that chiefly admit Na^+ [73,74].

Some cell types appear to exhibit two types of SOCs [25,75,76]. However, further experiments may be necessary to confirm that each type of SOC is, indeed, activated by a decrease in $[Ca^{2+}]_{er}$. At present, it can be concluded that there are several types of SOCs with different specificities for Ca^{2+}; however, with the exception of those Na^+ channels that are activated by a decrease in $[Ca^{2+}]_{er}$, SOCs principally conduct Ca^{2+} and hence might be expected to play roles in intracellular Ca^{2+} homoeostasis, as originally proposed by Putney [1,68].

Cation selectivity of animal cell RACCs

As described above, evaluation of the ability of a given RACC to admit Na^+ and other univalent cations, as well as Ca^{2+} (usually assessed by electrophysiological techniques), generally allows the RACC to be classified as a 'Ca^{2+} channel' or a 'non-specific cation channel'. In addition to Ca^{2+}, many RACCs admit the bivalent cations Mn^{2+}, Ba^{2+} and Sr^{2+} [77–82]. Since the first observation that RACCs admit Mn^{2+} [77–80], many RACCs have been tested for their ability to admit this cation, whereas tests of permeability to Ba^{2+} and Sr^{2+} have been less frequent. Ca^{2+} inflow through RACCs is often blocked by bi- and tervalent ions such as Ni^{2+}, Zn^{2+} and Gd^{3+} (reviewed in [11,12,83]). Knowledge of ion selectivity and the action of blocking ions provides a potential means of defining different RACC subtypes (reviewed in [3,11,12]).

As mentioned above, CRACs admit bivalent cations in the following order of permeability: $Ca^{2+} > Ba^{2+} (= Sr^{2+}) \gg Mn^{2+}$ [11,12,16–18,72]. Other SOCs that have been identified in patch-clamp experiments as having Ca^{2+}-release-activated currents, such as those in *Xenopus* oocytes, have slightly different relative permeabilities for bivalent cations [71]. CRACs are blocked by the bivalent cations Zn^{2+}, Cd^{2+}, Be^{2+}, Co^{2+}, Mn^{2+}, Ni^{2+} and Ba^{2+}, and by the tervalent cations La^{3+} and Gd^{3+} (reviewed in [11,12,16–18]). These ions may block Ca^{2+} inflow by binding tightly to Ca^{2+}-binding sites in the channel pore or, in the case of Mn^{2+}, Ba^{2+} and Sr^{2+}, by moving through the channel at a substantially lower rate than Ca^{2+} [12].

Studies using fluorescent Ca^{2+} indicators have shown that most [22,78,80,83–85], but not all [81,83,84], SOCs identified using this experimental protocol admit Mn^{2+}, Ba^{2+} and Sr^{2+} as well as Ca^{2+}. Studies with some putative SOCs indicate that they also admit Co^{2+}, Zn^{2+}, Ni^{2+} and Cd^{2+} [85,86]. Ca^{2+} inflow through most SOCs is blocked by Gd^{3+}, La^{3+} and Ni^{2+} [83,85]. A number of other types of RACC (most likely intracellular-messenger-activated non-selective cation channels) have also been shown to admit Mn^{2+}, Ba^{2+} and Sr^{2+} [58,82,83,88], but for some RACCs in this category there is no evidence that Mn^{2+} is admitted [22,83,87,88]. For those putative intracellular-messenger-activated non-selective cation channels where Ba^{2+} inflow has been measured using electrophysiological techniques, the permeability for Ba^{2+} is found to be similar to that for Ca^{2+} (reviewed in [3,12]). Some [54,83], but not all [54,74,83], putative intracellular-messenger-activated non-selective cation channels are blocked by Gd^{3+} and La^{3+}.

It is difficult to compare the abilities of different SOCs and other RACCs to admit Mn^{2+}, Ba^{2+} and Sr^{2+} other than qualitatively. This is because quantitative measurement of bivalent-cation inflow, and a quantitative comparison of rates of bivalent-cation inflow between different RACCs measured using fluorescent indicators, is difficult. Moreover, the rate of flow of a bivalent cation through a given RACC is greatly influenced by the experimental extracellular and intracellular ionic conditions employed [7,11,12].

Patch-clamp electrophysiological measurements have provided the most reliable quantitative data for bivalent-cation inflow through RACCs (reviewed in [12]). These data are usually in the form of the relative permeability of the different cations determined under a particular set of experimental conditions (including temperature and the ionic composition of the media) [12,18]. The use of intracellular fluorescent Ca^{2+} indicators, such as fura-2, for the measurement of Ba^{2+}, Sr^{2+} and Mn^{2+} inflow

[77–82] provides a general indication that a given RACC admits a particular bivalent cation. However, there have only been a few instances where bivalent-cation inflow measured using fluorescence indicators has been quantified [78]. Moreover, interpretation of the results obtained for Mn^{2+} inflow requires accurate knowledge of the intracellular location of the fluorescent dye [89,90].

It can be concluded that, while bivalent-ion selectivity and the ability of bi- and ter-valent ions to block Ca^{2+} inflow through RACCs are potentially valuable aids in distinguishing different types of RACCs, quantitative knowledge of these properties and the correlation of these ion-permeability properties with other properties of RACCs is still evolving. In the future, this knowledge, together with that of the key amino acid sequences that line the channel pore, should assist in understanding the properties of different RACCs and in distinguishing their characteristics.

TRP Ca^{2+} channels and TRPL non-selective cation channels in the *Drosophila* photoreceptor cell

During the past 5 years, knowledge of the structures and likely functions of two proteins located in the photoreceptor cells of *Drosophila melanogaster* has had a profound influence in shaping the direction of research on the structures and mechanisms of activation of RACCs in animal cells [2,12–15]. These proteins are the TRP [91,92] and TRPL [93] proteins. In the context of a summary of the experimental observations that help to define the different types of animal cell RACCs, it is useful to briefly discuss the properties of TRP and TRPL both *in situ* (in the photoreceptor cell) and when expressed heterologously in other cell types.

TRP, a plasma-membrane Ca^{2+} channel, and TRPL, a plasma-membrane non-selective cation channel, are both located in the microvilli that comprise the rhabdomere of the *Drosophila* photoreceptor cell [15,94]. The absorption of light by rhodopsin leads to an increase in the open-probability of TRP and TRPL, the flow of Na^+ and Ca^{2+} into the cytoplasmic space and depolarization of the photoreceptor cell plasma membrane [15,94]. At the time that TRP and TRPL were discovered, it was also known that the absorption of light by rhodopsin leads to the activation of a phospholipase C isoform (encoded by the *Drosophila NorpA* gene) and the formation of $InsP_3$. $InsP_3$ was considered a possible activator of TRP and/or TRPL. It was proposed that $InsP_3$ either binds directly to the channel protein or acts indirectly through a postulated $InsP_3$-induced decrease in $[Ca^{2+}]_{er}$ [91–94]. In view of these ideas, there has been considerable interest in TRP and TRPL as possible models for SOCs and other RACCs in animal cells [2,12–15].

Experiments have been conducted with *Drosophila* photoreceptor cells, and with TRP and TRPL expressed heterologously in insect Sf9 cells, *Xenopus* oocytes and several types of mammalian cells, in order to determine how the TRP and TRPL channels are activated in the *Drosophila* photoreceptor cell (reviewed in [2,12–15,95,96]). However, elucidation of these activation mechanisms has not been easy. While there is some evidence suggesting that TRP is activated by a decrease in the concentration of Ca^{2+} in the lumen of the submicrovillar cisternae (the ER of the *Drosophila* photoreceptor cell) [97,98], this point has not been established and further experiments are required to test this idea [15]. Other experiments indicate that, in the photoreceptor cell: (1) $InsP_3$ may directly activate TRP and/or TRPL, (2) the opening of these channels can be regulated by protein kinase C (the *Drosophila InaC* gene product), and (3) Ca^{2+} and calmodulin are involved in the termination of activation (reviewed in [15]).

Two observations on the spatial arrangement of intracellular signalling pathways in the *Drosophila* photoreceptor cell are relevant to considerations of the properties and mechanisms of activation of TRP and TRPL. First, while TRP and TRPL are located in the microvilli of the photoreceptor cell [99], the submicrovillar cisternae are located some distance away in the cell body at the base of the rhabdomere (reviewed in [15,94–96,99]). Secondly, experiments from a number of laboratories have shown that the TRP protein interacts with an elaborate protein network consisting of the InaD 'scaffolding' protein [containing five PDZ (protein interaction module [96]) domains] to which is also attached phospholipase C (the NorpA protein), protein kinase C (the InaC protein) and calmodulin (reviewed in [15,95,96]).

STRUCTURES OF RACCs

General features of plasma-membrane Ca^{2+}-channel structure

Having summarized current knowledge that defines the RACC subtypes, it is now pertinent to consider the structures and mechanisms of activation of the RACCs. Considerable knowledge has accrued of the amino acid sequences and topologies of the pore-forming subunits of the VOCCs [9,10], the LGCCs [100], the cyclic-nucleotide-gated RACCs [30] and the *Drosophila* TRP and TRPL RACCs [15,91–93]. Conspicuously absent from this body of knowledge are clear definitions of the structures of the pore-forming subunits of the large majority of RACCs, including SOCs.

With the exception of the cyclic-nucleotide-gated non-specific cation channels and the *Drosophila* TRP and TRPL proteins, neither the protein nor the cDNA encoding the protein for any other RACC has been unequivocally identified. It is likely that the subunit structures and topologies of the pore-forming subunits of RACCs are broadly similar to those of VOCCs, LGCCs, cyclic-nucleotide-gated non-selective cation channels and *Drosophila* TRP and TRPL channels [12–15,101,102]. VOCCs and LGCCs are composed of several pore-forming subunits which are often associated with regulatory subunits [9,10,100]. The pore-forming subunit (polypeptide) comprises between four and six membrane-spanning sequences, a hairpin sequence which dips into the hydrophobic region of the membrane to create the pore, and one or more allosteric regulatory sites (e.g. the positively charged amino acids in the S4-membrane-spanning sequence of the α subunit of VOCCs) that mediate channel opening [9,10,100].

Cyclic-nucleotide-gated non-specific cation channels

Cyclic-nucleotide-gated non-specific cation channels are tetramers of pore-forming subunits (reviewed in [30,101–103]). There is evidence for the existence of two types of pore-forming subunits, α and β, and it is hypothesized that functional tetramers are formed both from identical subunits (homotetramers) and from a mixture of α and β subunits (heterotetramers). Each tetramer possesses four binding sites for cyclic nucleotides. Each pore-forming subunit is composed of an N-terminal domain, six membrane-spanning sequences which are similar to one motif of the α_1 VOCC polypeptide, and a C-terminal domain which creates the cyclic nucleotide binding site (reviewed in [30,102,103]) (Figure 4a). The channel pore is proposed to be created by the four hydrophobic S5–S6 regions, each of which is donated by one subunit of the tetramer. While the S4 membrane-spanning sequence of the pore-forming polypeptide is positively charged, this structural feature does not appear to render cyclic-

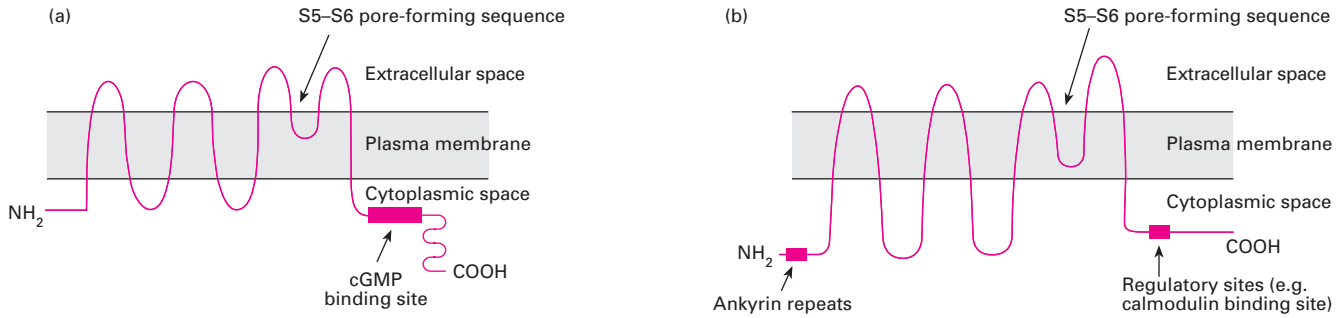

Figure 4 Topologies of the pore-forming subunits of a cyclic-nucleotide-activated non-selective cation channel (a) and the *Drosophila* TRP Ca^{2+} channel (b)

Table 1 Homologues of the *Drosophila* TRP Ca^{2+} channel detected in animal cells

TRP homologue	Species	Variants	Selected properties of channel expressed heterologously	References
TRP-1	Human	hTRP-1, hTRP-C1A, hTRP-C1	Non-specific cation channel; some evidence for activation by Ca^{2+} release from the ER	106–108,110
	Mouse	mTRP-1	Expression not yet reported	
	Xenopus laevis	x-TRP	Expression not yet reported	
TRP-2	Human	hTRP-C2	Expression not yet reported	13,15,107,111
	Mouse	mTRP-2	Expression not yet reported	
TRP-3	Human	hTRP-3, hTRP-C3	Non-specific cation channel; some evidence for activation by Ca^{2+} release from the ER	109,112–114
	Mouse	mTRP-3	Expression not yet reported	
TRP-4	Cattle	bCCE	Ca^{2+}-specific cation channel; some evidence for activation by Ca^{2+} release from the ER	115,116
	Human	hTRP-4	Expression not yet reported	
	Rat	TRP-R	Expression not yet reported	
	Mouse	mTRP-4	Expression not yet reported	
TRP-5	Mouse	mTRP-5	Ca^{2+}-specific cation channel activated by Ca^{2+}	117
	Rabbit	TRP-5	Evidence for activation by Ca^{2+} release from the ER	118
TRP-6	Mouse	mTRP-6	Non-specific cation channel; evidence for activation initiated by agonist–receptor complex	119
	Squid	sTRP	Expression not yet reported	120

nucleotide-gated channels responsive to membrane depolarization (reviewed in [30,102,103]).

Drosophila TRP and TRPL channels

The *Drosophila* photoreceptor TRP and TRPL channels are proposed to be homotetramers of TRP and TRPL pore-forming subunits respectively [13,15]. The possibility that heterotetramers of TRP and TRPL pore-forming subunits are also present in the photoreceptor cell has been considered, but the presence of heterotetramers in photoreceptor cells has not yet been demonstrated conclusively [15]. The overall structure of each of the TRP and TRPL pore-forming subunits (Figure 4b) is similar to that of one motif of the VOCC pore-forming α_1 polypeptide and to the cyclic-nucleotide-gated non-specific cation channel pore-forming subunit [13,15,93]. The N-terminal domain of TRP and TRPL contains ankyrin repeat sequences, and the C-terminal domain contains one (TRP) or two (TRPL) calmodulin-binding sites [13,15,93] (Figure 4b).

TRP homologues as models for RACCs

Progress in elucidation of the structures of the vast majority of RACCs, including the CRACs and other SOCs, has been slow. This is probably due to: (1) the likelihood that these proteins are expressed at quite low levels [12,18,72], and (2) the fact that no tight-binding inhibitors of these RACCs, which might be employed to isolate RACC proteins, are known. Most attempts at elucidation of the structures of RACCs have been based on the (initially) reasonable assumption that there are similarities between the sequences of the DNA encoding conserved regions of RACCs and the DNA encoding conserved regions of the known plasma-membrane Ca^{2+} channels and non-specific cation channels, namely the pore-forming subunits of *Drosophila* TRP and TRPL [13], the α_1 VOCC subunits [104] and the cyclic-nucleotide-gated channels [30]. Attempts have also been made to identify cDNAs encoding the pore-forming subunits of RACCs by expression cloning in *Xenopus* oocytes [105], but this approach has so far proved unsuccessful.

Since the emergence of the idea that the *Drosophila* TRP and TRPL cation channels are possible models for animal cell RACCs, considerable effort has been directed to determining whether homologues of TRP are present in animal cells, and whether these correspond to functional SOCs or to other RACCs. A number of TRP homologues, which are likely candidates for RACCs, have been identified (Table 1). The TRP-homologue approach has employed reverse-transcription–PCR and DNA primers (often degenerate) which are conserved regions of DNA encoding the *Drosophila* TRP channel, or in later studies conserved regions of previously identified animal cell TRP homologues (reviewed in [13,15]). Northern analysis has been used to determine the degree of expression of mRNA

encoding a given TRP homologue in different cell types (reviewed in [13,15]).

In general terms, the amino acid sequences and topologies of the TRP homologues are similar to those of *Drosophila* TRP and TRPL (Figure 4b). In order to determine whether a given TRP homologue is the pore-forming subunit of a RACC in the cell type under investigation, two approaches are being employed (reviewed in [13,15]). In the first, the ability of antisense DNA directed against a region of a given TRP homologue DNA to abolish receptor- or store-activated Ca^{2+} inflow, or a component of this Ca^{2+} inflow, is tested. In the second approach, the TRP homologue is expressed heterologously in another cell type (preferably one with low endogenous RACC activity) and the properties of the expressed TRP-homologue cation channel are compared with those of endogenous RACCs in the cell type under investigation. TRP-1 and TRP-3–TRP-6 have been expressed in this way (Table 1).

Interpretation of the results of experiments employing these two approaches has not been easy. The reasons for this include the low expression of endogenous RACCs in animal cells, the presence of multiple types of RACC in a given cell type and thus the need to identify the endogenous RACC subtype of interest against this background, difficulties in completely suppressing the expression of a given endogenous RACC using antisense DNA, the presence of endogenous RACCs in host cells used for heterologous expression of a TRP homologue, difficulties in comparing the properties of TRP-homologue Ca^{2+} channels expressed heterologously with those of the endogenous RACCs in the cell type under study, uncertainties about the mechanisms of activation of RACCs, and the difficulty (described above) of distinguishing SOCs from Ca^{2+}-activated non-selective cation channels. Interpretation of the results is also complicated by the possibility that a given TRP homologue polypeptide may combine with another (different) TRP-homologue polypeptide to form heterotetramers which are functional Ca^{2+} channels or functional non-selective cation channels [15].

Despite these difficulties, some deductions can be made from the results obtained so far. First, it is a reasonable conclusion that the TRP homologues identified to date in animal cells do represent functionally important (but not yet fully understood) receptor-activated Ca^{2+} and non-selective cation channels [13,15,117]. It is noteworthy that a number of these TRP homologues appear to be activated by Ca^{2+} (Table 1). Secondly, while some evidence has been presented to suggest that hTRP-1, hTRP-3 and hTRP-4 (where h denotes human) and rabbit TRP-5 may be SOCs (i.e. may be activated by a decrease in $[Ca^{2+}]_{er}$) (Table 1), further experiments are required to test this hypothesis. Thirdly, it appears that none of the TRP homologues so far identified has ion-selectivity properties similar to those of the CRACs, although the possibility that there are TRP homologue heterotetramers with the cation specificity of CRACs has not been eliminated [15]. Thus it is likely that at least one group of animal cell RACCs, the CRACs, are members of a family of channels with amino acid sequences that are somewhat different from those of the TRP homologues.

A second approach employed to determine the structures of CRACs and other Ca^{2+}-specific SOCs has been based on the idea that a common feature of VOCCs and CRACs is their high specificity for Ca^{2+}. Thus it might be expected that regions of the amino acid sequences of the pore-forming subunits of Ca^{2+}-specific SOCs are similar to sequences of the α_1 subunits of VOCCs. So far, reverse transcription–PCR strategies based on this approach have not yielded putative candidates for SOCs [104]. However, it is of interest to note that RACCs that are sensitive to inhibitors of L-type VOCCs, but are not activated by membrane depolarization, have been detected in several types of animal cells [121–124]. Moreover, functional L-type VOCCs have been detected in some non-excitable cells [125–129], and some L-type VOCC isoforms or homologues, which are unlikely to exhibit the normal functions of VOCCs, have been detected in a number of animal cell types [104,130]. Furthermore, VOCC homologues have been detected in yeast, and there is evidence that they are responsible for a physiological Ca^{2+} inflow [131,132].

MECHANISMS OF ACTIVATION OF RACCs

Intracellular-messenger- and Ca^{2+}-activated non-specific cation channels

With the exception of cyclic-nucleotide-gated non-specific cation channels and emerging knowledge of the regulation of *Drosophila* TRP and TRPL by Ca^{2+}, little is known of the mechanisms of activation of intracellular-messenger- and Ca^{2+}-activated non-specific Ca^{2+} channels. The mechanism of opening of cyclic-nucleotide-gated non-specific cation channels may serve as a model which can be used as the basis for elucidation of the mechanism of activation of other intracellular-messenger-activated non-specific cation channels when the structures and activators of these channels have been defined more clearly. The binding of cGMP or cAMP to the cyclic nucleotide binding site on the cytoplasmic face of a cyclic-nucleotide-activated non-specific cation channel protein alters the conformation of the protein and increases the probability of channel opening [30,102]. As is the case for LGCCs, cyclic-nucleotide-activated non-specific cation channels are thought to undergo a series of conformational changes which are required for transition of the channels between the open and closed states [30,102].

Although a number of Ca^{2+}-activated non-selective cation channels have been identified in animal cells [49–54], the mechanisms by which the binding of Ca^{2+} increases the probability of channel opening are not well understood. Activation by Ca^{2+} may involve the binding of Ca^{2+} directly to a site on the channel protein, or the action of Ca^{2+} may be mediated by a Ca^{2+}-binding protein such as calmodulin. In the case of the *Drosophila* TRPL non-selective cation channel, the binding of calmodulin to one of two calmodulin-binding sites permits the activation of TRPL by concentrations of Ca^{2+} in the range of physiological $[Ca^{2+}]_c$ [46,133,134], whereas there is evidence that the binding of calmodulin to the other calmodulin-binding site leads to inhibition of channel opening [46,134].

It is possible that, in some cases, the activation of Ca^{2+} inflow attributed to the opening of RACCs may reflect the opening of VOCCs by a process that does not involve depolarization of the plasma membrane. Mechanisms that may achieve this include the interaction of G-protein subunits with the VOCC α_1 subunit [21,135] and phosphorylation of the VOCC α_1 subunit [136–139].

SOCs

Despite the importance of SOCs for the generation of intracellular Ca^{2+} signals, the wide distribution of SOCs among different types of animal cells and the considerable effort that has been directed towards elucidation of the mechanisms by which SOCs are activated, these mechanisms are presently not well understood [1–3,12–15,140]. The question being addressed by hypotheses that seek to explain how SOCs are activated is: how does a decrease in $[Ca^{2+}]_{er}$ in the lumen of the ER lead to an increase in the probability of opening of SOC proteins located in the plasma membrane? In considering this question, it is useful to attempt to distinguish between those processes, events and regulatory proteins that are likely to be directly involved in the activation

Table 2 Current hypotheses for the mechanism(s) of activation of SOCs

Hypothesis	Brief description	Comments
Conformational coupling	It is hypothesized that there is protein–protein interaction between the InsP_3-receptor Ca^{2+}-channel protein (possibly the type 3 InsP_3 receptor Ca^{2+} channel) or another protein in the ER, and the SOC protein in the plasma membrane [1,2,42,47,141].	Recent experiments suggest that thapsigargin activation of SOCs is independent of InsP_3 receptor Ca^{2+}-channel proteins [142,143]. However, interactions between SOC proteins and another ER protein are possible.
Mobile intracellular messenger	It is proposed that a mobile intracellular messenger is released from the ER, binds to the SOC protein and increases probability of opening of the SOC [144,145]. Proposed messengers include Ca^{2+}-influx factor (CIF) [144], cytochrome *P*-450 metabolites (reviewed in [146]) and arachidonic acid generated by the action of phospholipase A_2 [48].	No clear evidence yet for a common (universal) mobile intracellular messenger with the required properties [2,12].
Vesicle insertion hypothesis	It is hypothesized that there is a reservoir of SOC channel proteins in intracellular vesicles. The activation of SOCs involves insertion of new SOC proteins into the plasma membrane [147].	Some evidence suggests that this hypothesis may be unlikely, or at least not universal [148].
G-protein coupling	It is proposed that a monomeric or trimeric G-protein couples the decrease in $[Ca^{2+}]_{er}$ to activation of SOCs [56,63–66].	While G-proteins are required for the activation of SOCs in some cell types, it is more likely that they play a permissive role in SOC activation [149].
Type 3 InsP_3 receptor	It is proposed that, in some cells, SOCs are type 3 InsP_3 receptors. Activation would require the binding of InsP_3 with low affinity, and regulation of this InsP_3 binding by the decrease in $[Ca^{2+}]_{er}$ [42].	Further experimental tests are required.
Decrease in $[Ca^{2+}]$ in the sub-plasmalemmal space	It is proposed that the decrease in $[Ca^{2+}]_{er}$ activates SERCAs, and that activated SERCAs decrease $[Ca^{2+}]$ in the sub-plasmalemmal space which, in turn, de-inhibits (activates) SOCs [67,68,140].	Not yet tested directly.
Direct connection between extracellular space and ER	A tube-like connection between the plasma membrane and the ER, involving endocytotic and exocytotic organelles, is proposed [150] (cf. [68]).	Not yet tested directly.

pathway, and those that may have a permissive role [12]. The current hypotheses for the mechanism of activation of SOCs will be considered first, with a brief consideration of possible permissive processes at the end of this section.

The various hypotheses that have been proposed to explain how SOCs are opened are summarized in Table 2. In addition to the ideas listed in Table 2, it is also possible that the decrease in $[Ca^{2-}]_{er}$ is a permissive event that is a prerequisite for the opening of SOCs by another, presently undefined, pathway. The two hypotheses for the mechanism of activation of SOCs that are presently considered to be most likely and that have received most attention are the 'conformational coupling' [47,141] and the 'mobile intracellular messenger' [144,145] hypotheses. Although a number of recent experiments have been directed towards testing these hypotheses, the results have proven inconclusive. These recent experiments have highlighted the difficulties involved in testing any hypothesis about the activation of SOCs [151,152].

While direct tests of the various hypotheses for the activation of SOCs have proven difficult, progress has recently been made in attempting to define the regions of the ER involved in the activation of SOCs, and the roles of InsP_3-receptor Ca^{2+} channels in these regions of the ER. Two different experimental approaches have shown that, under certain experimental conditions, Ca^{2+} can be released from a considerable proportion of the ER without the detectable activation of SOCs [153,154]. Comparison of the abilities of InsP_3, thapsigargin and ionomycin to release Ca^{2+} from the ER and open CRACs in patch-clamp experiments with rat basophilic leukaemia (RBL) cells has led to the conclusion that a small region of the ER, presumably located near the plasma membrane, is chiefly responsible for the activation of CRACs [153]. This region is characterized by an apparently lower affinity for InsP_3 than that of the bulk of the ER [153].

The theory that a subregion of the ER is specifically involved in the activation of SOCs is consistent with the results of experiments conducted with *Xenopus* oocytes in which the InsP_3-receptor Ca^{2+} channel agonist adenophostin A has been used to induce the opening of SOCs [155,156]. The concentration of adenophostin A that was found to give half-maximal release of Ca^{2+} from the bulk of the ER was significantly higher than that which induced the opening of SOCs [155,156]. While these results indicate that InsP_3-receptor Ca^{2+}-channel proteins play an important role in releasing Ca^{2+} from those regions of the ER hypothesized to be involved in the opening of SOCs, antisense techniques have been used to show that none of the type 1, 2 or 3 InsP_3-receptor Ca^{2+}-channel proteins are required to induce the opening of SOCs once Ca^{2+} has been released from the relevant region of the ER [142,143].

The idea that a specific region of the ER is involved in inducing the opening of SOCs is also consistent with the results of recent studies on the distribution of the various proteins involved in ER Ca^{2+} homoeostasis in different regions of the ER [157,158]. These experiments have revealed subregions of the ER that are distinguished by their enrichment with thapsigargin-sensitive or thapsigargin-insensitive SERCAs, InsP_3-receptor Ca^{2+} channels and ryanodine-receptor Ca^{2+} channels [157,158]. These attributes of the ER coexist with another property of the ER, continuity of the lumen. Recent experiments have confirmed that, within a given cell, the lumen of the ER is continuous, so that proteins and other molecules can diffuse to all parts of the ER lumen [159]. Thus it is necessary to reconcile the ability of Ca^{2+} to diffuse through the full extent of the lumen of the ER, on the one hand, with observed changes in $[Ca^{2+}]_{er}$ in distinct subregions of the ER and observations suggesting that a decrease in $[Ca^{2+}]_{er}$ in the peripheral ER can activate SOCs without an apparent decrease in $[Ca^{2+}]_{er}$ in the interior of the ER, on the other hand. Reconciliation of these facts may, in part, involve the likelihood that the apparent rate of diffusion of Ca^{2+} through the lumen of the ER is low, and that there may be particular spatial arrangements of subregions of the ER lumen.

Several proteins probably play a permissive role in the process by which a decrease in $[Ca^{2+}]_{er}$ leads to the opening of SOCs. These proteins include trimeric and monomeric G-proteins [56,63–66,149], Src and other protein-tyrosine kinases [160,161], and components of the cytoskeleton [162]. Although definitive evidence has not yet been obtained for such a role for any of these proteins, it has been shown that, in some types of cells, the functioning of a member of one or more of these groups of proteins is required for the activation of SOCs. It can be hypothesized that G-proteins and the cytoskeleton are required to maintain regions of the ER in an appropriate location near the plasma membrane, and/or to maintain uninterrupted communication within all regions of the lumen of the ER [163]. Further experiments are required to test this hypothesis and to extend knowledge of the role of the protein-tyrosine kinases.

PHYSIOLOGICAL FUNCTIONS OF RACCs

The need for several types of plasma-membrane Ca^{2+} channels in a single cell

The discussion above has emphasized the multiplicity of pathways which have evolved to allow Ca^{2+} present in the extracellular medium to enter the cytoplasmic space of animal cells. It is likely that the different Ca^{2+}-entry pathways (VOCCs, LGCCs and RACCs) have evolved in order to allow the generation of Ca^{2+} signals with different intensities, durations and spatial locations, and to accommodate the different intracellular sites of the enzymes and proteins that are the targets of Ca^{2+} action [4–6]. An indication of how the different plasma-membrane Ca^{2+} channels might generate different types of Ca^{2+} signals is shown in Figure 5.

The various plasma-membrane Ca^{2+} channels may also have evolved to accommodate the different intracellular structures that determine the fate of Ca^{2+} which enters the cytoplasmic space. These structures include the ER and the mitochondria [164–167] and other intracellular Ca^{2+} buffers [168], and restrictions imposed on the movement of Ca^{2+} within the cytoplasmic space by the membranes of the ER and other organelles, and possibly by the cytoskeleton [167,169]. A further reason for the variety of plasma-membrane Ca^{2+} channels may be to equip cells with a selection of channels that can be used to provide Ca^{2+} inflow under the different conditions in which the cell must normally function. For example, a number of agonists induce an alteration in membrane potential at the same time as creating an intracellular Ca^{2+} signal. The ways in which the various plasma-membrane Ca^{2+} channels contribute, or might contribute, to the generation of specific intracellular Ca^{2+} signals will now be considered.

VOCCs deliver large quantities of Ca^{2+} to specific locations in the subplasmalemmal space [9,10,170]. These locations are determined by the distribution of VOCCs in the plasma membrane [170]. The increase in $[Ca^{2+}]_c$ caused by the opening of VOCCs is generally restricted to a small region, sometimes called the Ca^{2+} domain (Figure 5a), of the cytoplasmic space in the vicinity of each VOCC. Restriction of the increase in $[Ca^{2+}]_c$ to this region is achieved by cytoplasmic Ca^{2+} buffers and by the rapid inactivation of open VOCCs [6,170,171]. Presumably the function of VOCCs in these environments is to deliver a rapid and large increase in $[Ca^{2+}]_c$ which activates Ca^{2+}-responsive target enzymes. VOCCs may also have other functions. For example, there is evidence that some VOCCs act to replenish ER Ca^{2+} stores [124,172,173].

The opening of LGCCs leads to the inflow of large amounts of both Na^+ and Ca^{2+} into the cytoplasmic space. Na^+ inflow causes depolarization of the plasma membrane, the generation of an action potential, and the opening of VOCCs which admit further amounts of Ca^{2+} (shown in Figure 5b) [7,100,174,175]. The Ca^{2+} that flows through LGCCs causes a rapid and large increase in $[Ca^{2+}]_c$ which, either alone or together with the flow of Ca^{2+} through VOCCs, induces a cell response such as neurotransmitter release. Elucidation of the physiological functions of LGCCs in non-excitable cells awaits further knowledge of the relative amounts of Na^+ and Ca^{2+} admitted to the cytoplasmic space through the open channels, the role of Na^+ entry in inducing plasma-membrane depolarization, and more complete knowledge of other possible intracellular effects of an increased cytoplasmic free Na^+ concentration ($[Na^+]_c$).

Intracellular-messenger-activated Ca^{2+} channels and non-selective cation channels

As implied above, intracellular-messenger-activated Ca^{2+} and non-selective cation channels may be more prevalent in animal cells than previously thought. The physiological roles of the cyclic-nucleotide-gated non-selective cation channels of mammalian sensory cells and of the TRP Ca^{2+} channel and the TRPL non-selective cation channel of the *Drosophila* photoreceptor cell have been reasonably well established. However, the contributions that the majority of these channels make to the generation of an intracellular Ca^{2+} signal, and possibly to other signalling pathways emanating from changes in $[Na^+]_c$, are not well understood.

In mammalian retinal, olfactory and gustatory cells, the flow of Na^+ and Ca^{2+} through cyclic-nucleotide-gated non-specific cation channels induces changes in the membrane potential. The closing of these channels in response to the absorption of light by rhodopsin, and the subsequent decrease in the concentration of cGMP, leads to hyperpolarization of the plasma membrane and, ultimately, to a decrease in the release of glutamate and other neurotransmitters [30,102].

While it has not been established unequivocally how TRP and TRPL are opened and how the opening of these channels is regulated in the *Drosophila* photoreceptor cell [15], there is considerable evidence that TRPL is opened by the actions of intracellular messengers (reviewed in [15]), and it is also likely that intracellular messengers regulate the opening of TRP [15]. Changes in $[Ca^{2+}]_c$ probably regulate both TRP and TRPL [15,46,133,134,176]. As is the case for the cyclic-nucleotide-gated non-specific cation channels in the mammalian retina, the flow of Na^+ and Ca^{2+} through the TRP and TRPL channels regulates photoreceptor-cell membrane potential [94]. However, in the *Drosophila* photoreceptor cell the absorption of light induces opening of the channels, enhanced inflow of Na^+ and Ca^{2+}, and depolarization of the plasma membrane. This, in turn, may open VOCCs that admit Ca^{2+} [94]. It is hypothesized that Ca^{2+} which enters the photoreceptor cell through TRP and TRPL channels, together with that which enters through VOCCs, leads to the release of neurotransmitters [94]. Thus it can be seen that one of the important functions of at least two types of RACC is to admit Na^+ rather than Ca^{2+}, so that functionally these might be regarded more as Na^+ channels than as Ca^{2+} channels.

The physiological functions, including the relative amounts of Na^+ and Ca^{2+} conducted through the channel, of the other intracellular-messenger-activated non-selective Ca^{2+} channels may depend on the channel subtype and the type of cell in which it is expressed. Thus it has been estimated that, in mast cells under physiological conditions, only a small proportion of Ca^{2+} inflow is mediated by non-selective cation channels activated by GTP[S] (putative intracellular-messenger-activated channels), while most Ca^{2+} inflow occurs through SOCs [24]. On the other

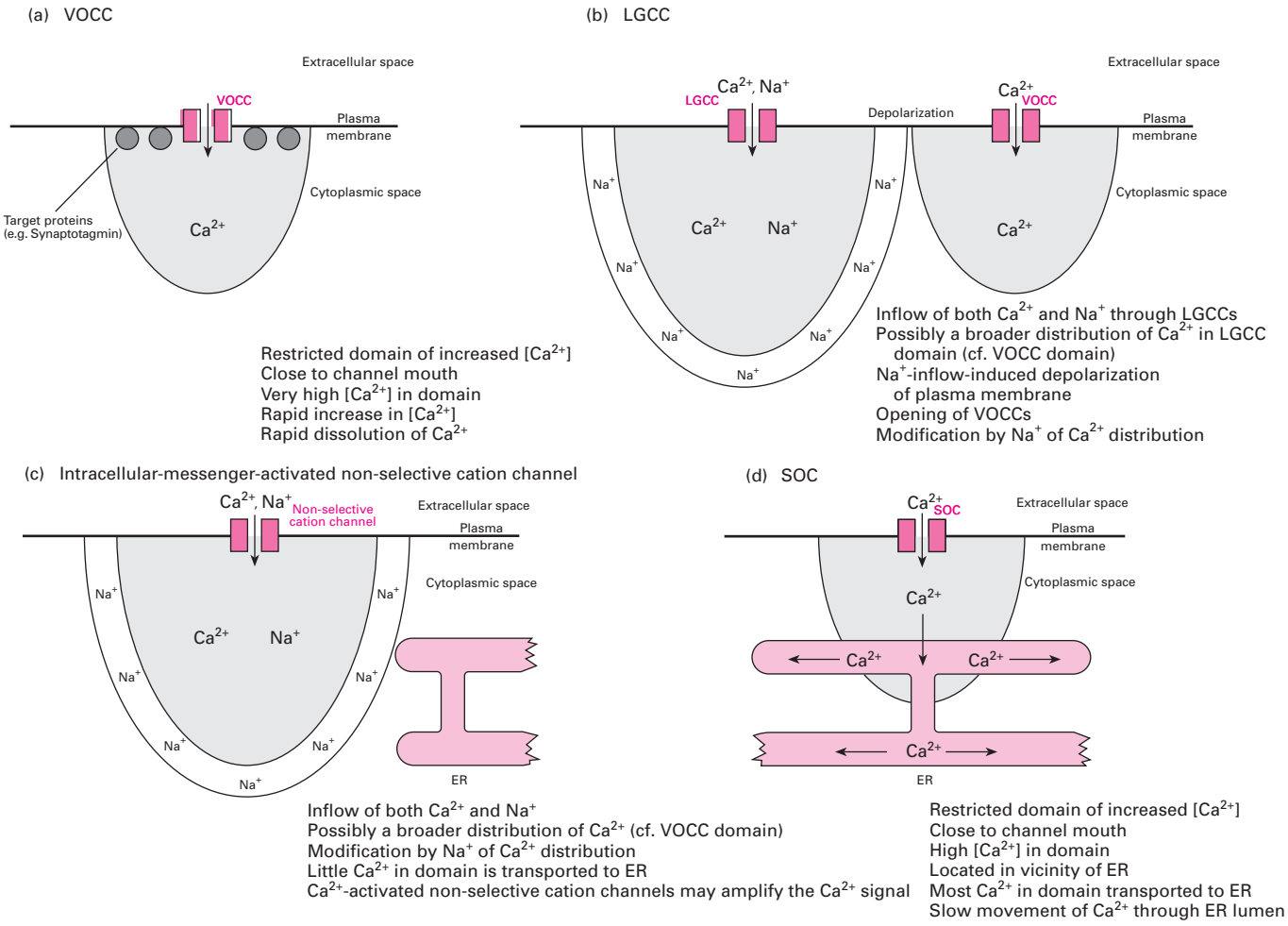

Figure 5 Different types of Ca^{2+} signals initiated by the opening of plasma-membrane Ca^{2+} channels

The nature of the Ca^{2+} signal generated by the opening of a given type of channel (shown by the light-grey domains and the presence of Ca^{2+} and Na^{+} ions) is determined by several factors, including: (1) the number of Ca^{2+} ions which flow through a single channel during the open period, (2) the time for which the single channel is open, (3) the rate at which the channel is opened and closed, (4) the density of channel proteins on the cell surface, (5) whether Na^{+} also flows through the open channel, (6) the proximity of the channel to the ER, mitochondria or other organelles that can take up Ca^{2+}, and (7) the proximity of the channel to other cellular structures that determine the apparent rate of diffusion of Ca^{2+} through the cytoplasmic space.

hand, evidence has been presented to indicate that an arachidonic-acid-activated Ca^{2+} channel makes a major contribution to Ca^{2+} inflow to human embryonic kidney cells expressing M3 muscarinic receptors under physiological conditions [23].

It can be hypothesized that intracellular-messenger-activated non-selective cation channels serve the following functions (shown in Figure 5c): (i) the facilitation of Ca^{2+} inflow to the cytoplasmic space and, possibly, to intracellular stores [23,24]; (ii) the facilitation of Na^{+} inflow to the cytoplasmic space. The increase in $[Na^{+}]_c$ leads in turn to depolarization of the plasma membrane, to the opening of VOCCs (and hence Ca^{2+} inflow), and possibly to the opening of other types of plasma-membrane voltage-operated ion channels [52,177–179]. The increase in $[Na^{+}]_c$ may also have effects on cell function that are independent of the consequences of plasma-membrane depolarization. For example, Na^{+} may alter the uptake of Ca^{2+} by mitochondria [180] and activate the plasma-membrane Na^{+}–Ca^{2+} exchanger [58,180,181]. Both of these systems have been shown to play important roles in determining the intracellular Ca^{2+} signal [58,164–167,180,181].

It is possible that, under some circumstances, Ca^{2+}-activated non-selective cation channels form part of a 'feed-forward' mechanism which amplifies the increase in $[Ca^{2+}]_c$ initiated by the release of Ca^{2+} from the ER. If this is the case, the flow of Ca^{2+} through these plasma-membrane channels must be very tightly regulated in order to prevent uncontrolled Ca^{2+} inflow to the cytoplasmic space.

SOCs

The idea that the predominant role of SOCs is the replenishment of Ca^{2+} in the ER

Since SOCs are activated by a decrease in $[Ca^{2+}]_{er}$, and since it was originally postulated that their function is to replenish Ca^{2+} in intracellular stores, it seems unnecessary to consider whether this is the sole function of SOCs. However, as discussed previously, under some experimental conditions an increase in $[Ca^{2+}]_c$ can clearly be demonstrated when SOCs are opened (reviewed in [1]). Such an increase could result from the direct diffusion of Ca^{2+} from SOCs to the cytoplasmic space, or from the movement of Ca^{2+} from the cytoplasmic mouth of SOCs into the ER and its subsequent release into the cytoplasmic space.

Although this question has previously been addressed extensively [1,67,68], the way in which Ca^{2+} moves into the cell from SOCs under physiological conditions is still not clear. Moreover, in the light of knowledge of the existence of intracellular-messenger-activated non-specific Ca^{2+} channels, VOCCs and LGCCs, all of which admit Ca^{2+} into the cytoplasmic space which could, in turn, be taken up by the ER, it is reasonable to ask whether SOCs play a unique role in replenishing ER Ca^{2+} and whether this might not also be effectively accomplished by other plasma-membrane Ca^{2+} channels.

Three observations help to provide some direct confirmation that SOCs play a vital role in cell function. First, it has been observed that the lymphocytes of a patient with a form of T-cell immunodeficiency exhibit neither I_{CRAC} nor an agonist-stimulated increase in $[Ca^{2+}]_c$ when compared with lymphocytes from control subjects. These abnormalities are associated with impaired lymphocyte proliferation [182]. Secondly, after the chemical mutagenesis of a Jurkat human T-lymphocyte cell line, mutant clones were isolated which exhibited no detectable I_{CRAC} and no thapsigargin- or agonist (OKT3, a mouse monoclonal antibody against the T-lymphocyte CD3 plasma-membrane receptor)-stimulated Ca^{2+} inflow [183]. Thirdly, studies with several cell types suggest that cell differentiation is associated with an increase in the expression of SOCs [184–187]. While these observations provide either direct or indirect evidence that SOCs are essential for cell function, further experiments are required to identify which proteins (e.g. the SOC protein or a protein required for SOC activation) exhibit altered expression in the mutated cell lines and in differentiated cells, and to determine more precisely the consequences of the ablation of one or more types of SOC for the intracellular Ca^{2+} signal generated by an agonist.

Another observation may be relevant to consideration of the physiological role of SOCs. The events that follow the binding of a given agonist to a receptor which is coupled to the formation of $InsP_3$ (the activation of G_q and phospholipase Cβ, the synthesis of $InsP_3$, the release of Ca^{2+} from the ER, the opening of SOCs and an increase in $[Ca^{2+}]_c$ [1]) can be considered to be components of an unbranched signalling pathway. Within this pathway, the ER (upstream) might be regarded as a 'feed-forward' regulator of the SOCs (downstream), i.e. the SOCs provide a mechanism which ensures that the ER, which is being depleted of Ca^{2+} by $InsP_3$, is constantly being refilled with Ca^{2+}. Thus the activation of SOCs is closely linked to the decrease in $[Ca^{2+}]_{er}$ and the generation of $InsP_3$. The concominant activation of Ca^{2+}-activated non-selective cation channels by the increase in $[Ca^{2+}]_c$ may represent an amplification of the cytoplasmic Ca^{2+} signal. By contrast, the initiation by the same agonist of the opening of intracellular-messenger-activated non-selective cation channels generally involves the activation of a different G-protein and the utilization of a signalling pathway other than the $InsP_3$ pathway (e.g. a pathway involving the formation of arachidonic acid or diacylglycerol [21,23]). Since this second pathway does not involve a decrease in $[Ca^{2+}]_{er}$, opening of the intracellular-messenger-activated non-selective cation channel need not be linked to re-filling of the ER Ca^{2+} store.

Importance of the spatial location of SOCs on the cell surface

Knowledge of the spatial location (distribution on the cell surface) would help greatly in understanding the physiological function of SOCs. On the basis of the estimated single-channel conductance of CRACs, which (as described above) is very low compared with the conductance of other known plasma-membrane Ca^{2+} channels, and the measured whole-cell current attributed to Ca^{2+} inflow through CRACs in lymphocytes, Zweifach and Lewis [188] have estimated that the number of CRACs on the lymphocyte plasma membrane is about 10000 channels per cell. This corresponds to a density of about one channel every 0.5 μm of cell surface. Although the estimated number of CRACs per cell is much greater than the estimated number of VOCCs (100–1000 channels per cell) [12], it is likely that the density of CRACs, which may be distributed evenly on the cell surface, is low compared with the density of VOCCs, which are generally concentrated in specific regions of the cell surface [7,170].

Some indication that there is a specific spatial distribution of SOCs on the surface of cells in relation to intracellular structures has come from studies of the physiological consequences of Ca^{2+} inflow through SOCs [189–192]. The experiments have involved a comparison of the abilities of Ca^{2+} which enters the cytoplasmic space through SOCs and Ca^{2+} which is released from intracellular stores to activate or inhibit enzymes located on the cytoplasmic face of the plasma membrane. In one series of experiments, Cooper and colleagues [189,191] have shown that in C6-2B glioma cells, which normally express the Ca^{2+}-inhibitable adenylate cyclase isoform VI, and in HEK cells expressing an exogenous adenylate cyclase VI, the activity of adenylate cyclase is inhibited by the increase in $[Ca^{2+}]_c$ that follows the opening of SOCs. The opening of SOCs was induced by the addition of $Ca^{2+}{}_o$ to cells incubated previously in the presence of thapsigargin, but in the absence of added $Ca^{2+}{}_o$ [189,191]. No inhibition of adenylate cyclase VI was observed when $[Ca^{2+}]_c$ was elevated due to the release of Ca^{2+} from the ER or to non-specific Ca^{2+} inflow across the plasma membrane induced by ionomycin [189,191]. It was also shown that Ca^{2+}-activatable adenylate cyclases I and VIII are activated by Ca^{2+} inflow through SOCs, but not by Ca^{2+} released from intracellular stores or by treatment of the cells with ionomycin [190].

These results indicate that the Ca^{2+}-sensitive adenylate cyclases studied are located in the same region of the plasma membrane as are SOCs, so that the enzymes are affected by the elevated free Ca^{2+} concentration at the cytoplasmic mouths of the open SOCs. Alternatively, the enzymes may be affected by the increase in $[Ca^{2+}]_c$ that results from the diffusion of Ca^{2+} into the cytoplasmic space in the regions surrounding the open SOCs. Interpretation of these results may not be completely straightforward since, as discussed above, in the presence of thapsigargin there may be increases in $[Ca^{2+}]_c$ in regions of the cytoplasmic space where Ca^{2+} would not normally accumulate under physiological conditions. Notwithstanding this possibility, the results obtained with the Ca^{2+}-sensitive adenylate cyclase isoforms suggest there is likely to be a specific spatial location of SOCs on the plasma membrane.

SOCs may provide Ca^{2+} for distribution through the ER network

Several observations suggest that the physiological role of SOCs may be to discretely supply Ca^{2+} to the ER in order to maintain an adequate concentration of Ca^{2+} in the lumen of the ER for use elsewhere in the cell (shown in Figures 5d and 6). First, as mentioned above, in the absence of a SERCA inhibitor, Ca^{2+} inflow through SOCs replenishes Ca^{2+} stores in the ER with little observable increase in $[Ca^{2+}]_c$ ([193,194]; reviewed in [1]). Furthermore, it has recently been shown that in pancreatic acinar cells the flow of Ca^{2+} through SOCs can replenish ER Ca^{2+} stores even when $[Ca^{2+}]_c$ is 'clamped' at the 'basal' (resting) value [195]. These observations suggest that, under physiological conditions, re-filling of the ER by Ca^{2+} is a reasonably discrete process and does not involve substantial diffusion of Ca^{2+} derived from SOCs into the cytoplasmic space. Further indirect evidence for the idea

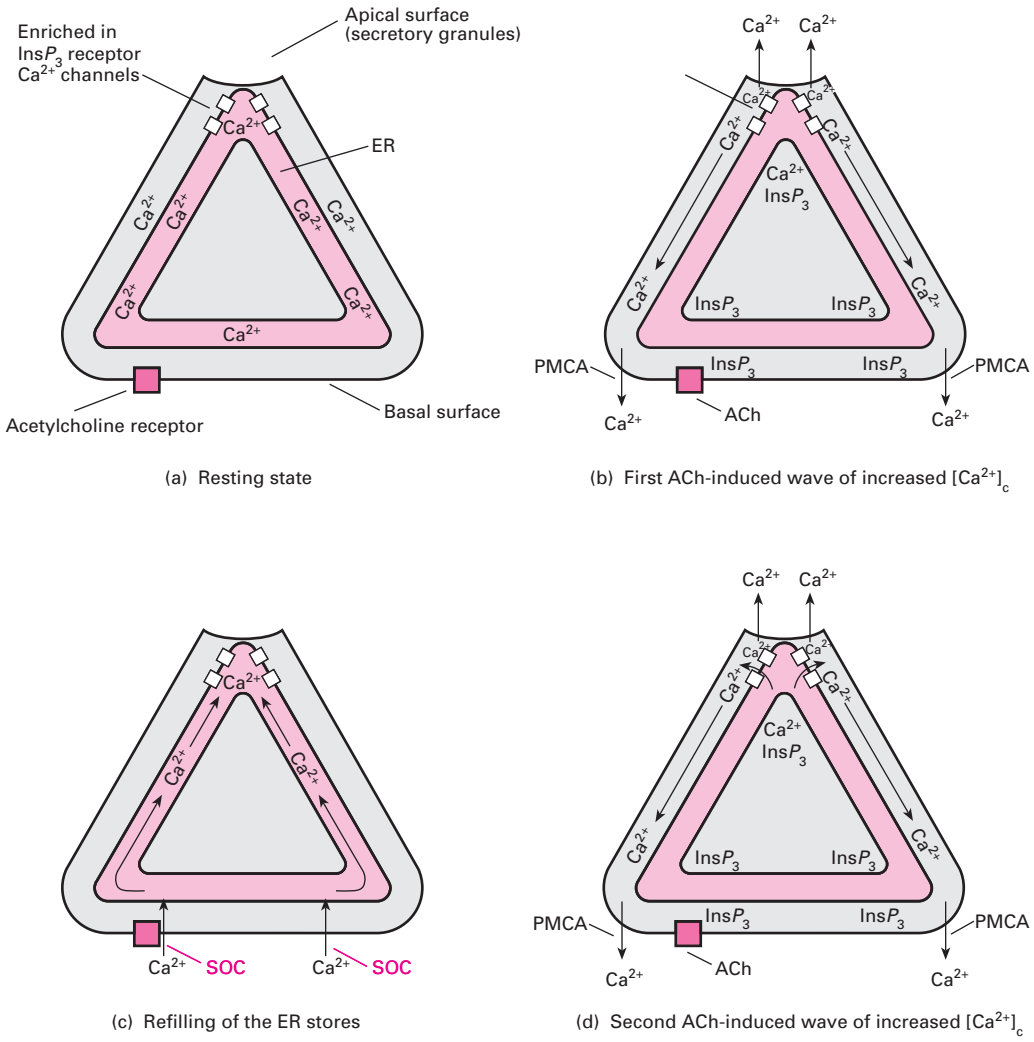

Figure 6 Hypothetical role of SOCs in replenishing ER Ca^{2+} stores in the pancreatic acinar cell

The representation shown was constructed on the basis of the results obtained by Mogami et al. [193,195], and the interpretation given by these authors of their own results. It is hypothesized that the binding of acetylcholine (ACh) to receptors on the basal surface (**b**) induces the formation of InsP_3, the diffusion of InsP_3 from the basal to the apical side of the cell, the release of Ca^{2+} through InsP_3-receptor Ca^{2+} channels which are predominantly located in the apical region, the initiation of a wave of increased $[Ca^{2+}]_c$ in the apical region and the propagation of this wave through the cytoplasmic space from the apical to the basal side of the cell (shown by the arrows in **b**). Some Ca^{2+} is pumped out of the cell by PMCAs at the basal surface (**b**). It is proposed that, when $[Ca^{2+}]_c$ is low (between two waves of increased $[Ca^{2+}]_c$), Ca^{2+} stores of the ER near the apical surface are replenished by the action of SOCs at the basal surface (**c**). The concentration of Ca^{2+} in the ER is then restored so that the next regenerative wave of increased $[Ca^{2+}]_c$ can proceed (**d**).

that there is limited diffusion of Ca^{2+} from the cytoplasmic mouths of SOCs comes from the observation that in HL-60 cells (a leukaemic neutrophil cell line) the increase in $[Ca^{2+}]_c$ induced by Ca^{2+} inflow through SOCs is insufficient to induce exocytosis, whereas the increase in $[Ca^{2+}]_c$ induced by Ca^{2+} inflow through VOCCs is effective in inducing exocytosis [192].

Secondly, Mogami et al. [193] have provided evidence indicating that Ca^{2+} admitted to the ER through SOCs located on the basal membrane of pancreatic acinar cells can move ('tunnel') through the lumen of the ER to the apical side of the cell (Figure 6). This conclusion that Ca^{2+} can move freely through the ER is consistent with recent observations mentioned above which confirm that the lumen of the ER is continuous [159].

Thirdly, many previous studies of the properties of SOCs have been conducted under non-physiological conditions in which $[Ca^{2+}]_o$ and $[Ca^{2+}]_{er}$ were reduced well below the physiological values (e.g. see [1] and [12] for reference to experimental protocols). It is likely that, under physiological conditions (normal $[Ca^{2+}]_o$) and at a physiological concentration of agonist, the intracellular Ca^{2+} signal in non-excitable cells is composed of repetitive waves of increased $[Ca^{2+}]_c$, or a relatively small increase in $[Ca^{2+}]_c$ which is sustained over a period of time [4,6,169,196]. There is evidence that, during each wave of increased $[Ca^{2+}]_c$, or during each sustained rise in $[Ca^{2+}]_c$, some Ca^{2+} is transported out of the cell via PMCAs (Figures 6b and 6d) [194,197–199]. Since, in the interior of the cell, Ca^{2+} released into the cytoplasmic space will be returned to the ER by the action of SERCAs, the consequence of the loss of Ca^{2+} to the extracellular medium is likely to be a decrease in $[Ca^{2+}]_{er}$ at the periphery of the cell.

Fourthly, current ideas for the mechanisms by which waves or oscillations of increased $[Ca^{2+}]_c$ move through the cell are based on evidence that the ER provides a network of stored Ca^{2+} which, for any particular location in the ER, acts a reservoir of Ca^{2+} that can be released into the cytoplasmic space at that

location in response to the actions of $InsP_3$ and Ca^{2+} at $InsP_3$-receptor Ca^{2+} channels, and the actions of Ca^{2+} and cyclic ADP-ribose at ryanodine-receptor Ca^{2+} channels [4,6,196]. The spatial locations of the ER and of ER Ca^{2+} channels are thought to determine the properties and direction of propagation of the wave of increased $[Ca^{2+}]_c$ [4,6,196].

On the basis of these observations, a reasonable hypothesis for the role of SOCs is that they deliver Ca^{2+} to the peripheral ER in order to maintain adequate concentrations of Ca^{2+} in the ER during the stimulation of cells by agonists that utilize $InsP_3$ as an intracellular messenger (Figures 5d and 6). This would enable the ER to carry out its role as a network of stored Ca^{2+} which is available for release to the cytoplasmic space when $InsP_3$-receptor and ryanodine-receptor Ca^{2+} channels open in specific regions of the ER. The concept that the SOCs discretely supply Ca^{2+} to the ER is consistent with experimental observations suggesting that Ca^{2+} inflow through SOCs 'drives' oscillations in $[Ca^{2+}]_c$ [12,169,199,200] (but see [23,201]).

In addition to the decrease in $[Ca^{2+}]_{er}$, which triggers the activation of SOCs, Ca^{2+} flow through SOCs is enhanced by hyperpolarization of the plasma membrane (reviewed in [12]). One mechanism by which hyperpolarization may be achieved is the opening of Ca^{2+}-activated K^+ channels [202]. Another, proposed by Hoth [203], is that ER Ca^{2+} depletion activates the outward movement of K^+ through CRACs themselves, and this, in turn, counteracts membrane depolarization caused by the inflow of Ca^{2+} through CRACs.

As mentioned above, the ability of Ca^{2+} to diffuse within the lumen of the ER [159,193] may allow Ca^{2+} to move from (1) a region of the ER in the cell interior to a region of the ER in the cell periphery during the time that Ca^{2+} is being released from the peripheral ER into the cytoplasmic space and transported out of the cell, and (2) from a region of the peripheral ER to a region of the interior ER during the time that Ca^{2+} inflow through SOCs replenishes Ca^{2+} in the ER. However, luminal Ca^{2+} movement is unlikely to be sufficiently rapid to contribute directly to the generation of waves of increased $[Ca^{2+}]_c$.

The arguments presented above suggest that SOCs play major roles in those cells with a reasonably complex intracellular structure and a ramifying ER network where it is necessary to feed Ca^{2+} into the ER network in order to maintain an adequate $[Ca^{2+}]_{er}$ for Ca^{2+}-wave propagation. This idea is consistent with the above-mentioned observation that cell differentiation, which might be expected to be accompanied by a more complex intracellular structure, is associated with an increased expression of SOCs [184–187]. The proposed function of SOCs differs from the functions proposed for intracellular-messenger-activated non-selective cation channels, which may contribute little Ca^{2+} for the refilling of ER Ca^{2+} stores and principally deliver Ca^{2+} directly to the cytoplasmic space (cf. Figures 5c and 5d).

CONCLUSIONS

The conclusions that can presently be reached concerning the various subtypes of RACCs, their structures and mechanisms of activation, and their physiological functions can be summarized as follows. (1) There appear to be two major subtypes of RACCs, the SOCs and the intracellular-messenger-activated non-selective cation channels, which admit Ca^{2+} as well as Na^+. (2) While the structures and details of the mechanisms of activation of the intracellular-messenger-activated non-selective cation channels are yet to be determined, it is likely that these will represent variations of the structural and functional elements employed by the cyclic-nucleotide-gated and the *Drosophila* TRP and TRPL cation channels. (3) Neither the structures nor the mechanisms of activation of SOCs (both the highly Ca^{2+}-selective CRACs and the less Ca^{2+}-selective subgroup of SOCs) have yet been uniquely defined. It is likely that these structures and activation mechanisms will be variations on the structural and functional elements of the known plasma-membrane Ca^{2+} channels. (4) A picture is beginning to emerge of how the properties (ion selectivity, mechanism of activation, location on the plasma membrane, kinetics of activation and de-activation) of the RACCs and other plasma-membrane Ca^{2+} channels fit a given subtype of Ca^{2+} channel to deliver a specific type of intracellular Ca^{2+} signal. (5) Fruitful areas of future investigation are likely to be elucidation of the structures and mechanisms of activation of RACCs, the role of a given subtype of RACC in generating the characteristic properties of a particular intracellular Ca^{2+} signal, and the spatial distribution of RACCs on the cell surface in relation to the ER and other intracellular structures.

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