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Review

Signaling pathways between the plasma membrane and endoplasmic reticulum calcium stores

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Abstract. This review discusses multiple ways in which the endoplasmic reticulum participates in and is influenced by signal transduction pathways. The endoplasmic reticulum provides a Ca^{2+} store that can be mobilized either by calcium-induced calcium release or by the diffusible messenger inositol 1,4,5-trisphosphate. Depletion of endoplasmic reticulum Ca^{2+} stores provides a signal that activates surface membrane Ca^{2+} channels, a process known as capacitative calcium entry. Depletion of endoplasmic reticulum stores can also signal long-term cellular responses such as gene expression and programmed cell death or apoptosis. In addition to serving as a source of cellular signals, the endoplasmic reticulum is also functionally and structurally modified by the Ca^{2+} and protein kinase C pathways. Elevated cytoplasmic Ca^{2+} causes a rearrangement and fragmentation of endoplasmic reticulum membranes. Protein kinase C activation reduces the storage capacity of the endoplasmic reticulum Ca^{2+} pool. In some cell types, protein kinase C inhibits capacitative calcium entry. Protein kinase C activation also protects the endoplasmic reticulum from the structural effects of high cytoplasmic Ca^{2+}. The emerging view is one of a complex network of pathways through which the endoplasmic reticulum and the Ca^{2+} and protein kinase C signaling pathways interact at various levels regulating cellular structure and function.

Key words. Calcium signaling; calcium pools; capacitative calcium entry; gene expression; apoptosis; endoplasmic reticulum structure; protein kinase C.

Introduction

All living cells have evolved means for regulating their content of calcium. This is because calcium is involved in signaling and regulatory control of cellular functions, and also because large and inappropriate changes in calcium can have deleterious effects on cell structure and function [1]. Since the discovery of the signaling role of calcium ions [2], the majority of research has focussed on regulation of calcium in the cytoplasm ($[Ca^{2+}]_i$) because therein reside the enzymes and other responsive proteins with which Ca^{2+} is thought to interact. From the early work, it was obvious that the source of Ca^{2+} for muscle contraction was derived largely from Ca^{2+} sequestered in intracellular organelles [3]. This storage site in muscle is the sarcoplasmic reticulum, a highly specialized organelle thought to be derived from endoplasmic reticulum [4].

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An understanding of the sites of Ca^{2+} mobilization in nonexcitable cells evolved more slowly, probably due to the lack of a readily quantifiable Ca^{2+}-dependent parameter analogous to muscle shortening to relate to $[Ca^{2+}]_i$ changes. Eventually, a general concept was put forth according to which cellular activation involved Ca^{2+} release from intracellular sites, as well as entry of Ca^{2+} across the plasma membrane [5]. It is interesting that despite the obvious analogy with signaling in muscle cells, a good deal of attention was paid to the mitochondria as potential sources of this signaling Ca^{2+}. However, it soon became apparent that the site of Ca^{2+} storage involved in stimulus-induced intracellular Ca^{2+} release was the endoplasmic reticulum, or a specialized component of it [6–9]. Thus, the simplest and most obvious function of endoplasmic reticulum Ca^{2+} stores is to provide a source for the intracellular release of Ca^{2+} to the cytoplasm in the course of Ca^{2+}-dependent signaling processes. However, recent findings in a number of somewhat divergent areas have indicated that the relationship of endoplasmic reticulum Ca^{2+} stores to signaling pathways is much more complex (for an excellent review of some of these complexities, see [10]). This relationship now appears to function as a two-way street: the endoplasmic reticulum provides and regulates cellular signaling pathways, but the structure and function of the endoplasmic reticulum Ca^{2+} stores are also influenced by cellular signals in ways that are not yet fully understood. The focus of this review is this reciprocal regulation that can occur between signaling pathways and the endoplasmic reticulum Ca^{2+} stores.

Signaling functions of endoplasmic reticulum calcium stores

Mechanisms of intracellular Ca^{2+} release

Research into mechanisms of calcium signaling by the endoplasmic reticulum has followed two tracks, one involving excitation-contraction in muscle, and the other calcium release by inositol 1,4,5-trisphosphate (IP_3) and other cellular mediators. The specialized endoplasmic reticulum of muscle, the sarcoplasmic reticulum, has long been known to show the property of calcium-induced calcium release (CICR) [4, 11]. That is, under the appropriate conditions, calcium itself can activate the release of additional calcium. This finding led to the concept of 'trigger' calcium, a small increment of calcium which would touch off the all-or-none regenerative CICR process. In the case of heart muscle, it appears that this trigger calcium comes from calcium influx through voltage-activated calcium channels as a result of the automatic, paced cardiac action potential. This theme probably occurs in a number of excitable cell types whereby Ca^{2+} entry and Ca^{2+} release are coordinated through an amplification of entry by intracellular CICR (fig. 1). In the case of skeletal muscle, voltage-dependent calcium channels in the t-tubule may also provide the triggering signal, but it is believed that this occurs by a relatively direct, physical interaction between the channel proteins and the calcium channels in the sarcoplasmic reticulum [11]. These sarcoplasmic reticulum calcium channels were first purified on the basis of their high binding affinity for the toxin ryanodine, and thus are generally referred to as ryanodine receptors [12, 13].

In nonmuscle cells, CICR may also contribute to the generation of calcium signals, but especially in electrically nonexcitable cells, the initiation of calcium signaling usually involves the chemical mediator IP_3 [14]. The IP_3 receptor is similar in structure to the ryanodine receptor, and like the ryanodine receptor exhibits CICR behavior under appropriate conditions [14–16]. There is growing evidence for the presence of ryanodine receptors in nonexcitable cells, and in such cases the activity of the ryanodine receptors may be controlled by another chemical mediator, cyclic ADP-ribose [17–19]. The regenerative calcium signaling properties of ryanodine and IP_3 receptors give rise to the characteristic intracellular calcium ($[Ca^{2+}]_i$) waves and oscillations seen in many excitable and nonexcitable cells [20, 21]. These digital $[Ca^{2+}]_i$ signals play important roles in providing high signal-to-noise inputs controlling short-term responses, such as secretion [22, 23], and long-term responses, such as gene expression [24, 25].

Control of calcium entry by the endoplasmic reticulum

Calcium signaling by the endoplasmic reticulum is intimately related to calcium fluxes across the plasma membrane. In many excitable cell types, the heart for example, the influx of calcium through calcium channels provides an initial calcium signal that is amplified through CICR from the endoplasmic or sarcoplasmic reticulum. In most nonexcitable cells, however, this interaction occurs in precisely the reverse order (fig. 1). The release of calcium from the endoplasmic reticulum provides a signal for the opening of calcium channels in the plasma membrane. This process, termed 'capacitative' calcium entry [26–28] or 'store-operated' calcium entry [29], does not, however, involve an activation of calcium channels by calcium. Rather, the signal is unknown but presumably involves either the release of some substance from the endoplasmic reticulum when it is depleted of calcium, or alternatively involves the interaction of endoplasmic reticulum proteins with plasma membrane calcium channels [28, 30].

Clues about the identity of the channel molecules responsible for capacitative calcium entry have come

from a *Drosophila* photoreceptor mutant, *trp*. This mutation results in a photoreceptor response that is transient rather than sustained, and since invertebrate photoreceptor signaling is believed to involve phospholipase C and IP_3, a role for TRP (transient receptor potential) protein in the sustained, calcium entry response was suggested [31]. That TRP actually functions as a capacitative calcium entry channel in the *Drosophila* photoreceptor has been seriously questioned [32]. Nonetheless, in exogenous expression systems, TRP appears capable of functioning as a component of a capacitative calcium entry channel [33–35].

A search for homologs of TRP in vertebrate cells has revealed a new family of putative ion channels which may play a role in capacitative calcium entry [36, 37]. Zhu et al. [38] reported full or partial sequences for six different mammalian TRP proteins, designated TRP1–6, and recently a seventh member of the family (TRP7) has been cloned [39]. These proteins fall into three or four categories based on structural similarities. TRP3, TRP6 and TRP7 are similar in structure, as are TRP4 and TRP5. Structurally similar proteins may thus subtend identical or similar functions. In humans, but not other mammalian species, TRP2 is a pseudogene (the mouse TRP2 gene has been functionally expressed; see [40]). Thus, in humans at least, only three distinct proteins are known: TRP1, TRP3/6/7 and TRP4/5. The cellular functions of these proteins have been studied primarily by their expression (or overexpression) in cells. To date, these have involved cell types which already express capacitative calcium entry, likely a necessity in order for the signaling to occur as a result of the depletion of endoplasmic reticulum calcium stores. The behavior of cells overexpressing TRP proteins varies depending on the form of TRP expressed, but also among different laboratories for reasons that are not clear. TRP3 was the first member of the mammalian TRP family to be expressed and shown to augment calcium signals [38]. Its overexpression augmented $[Ca^{2+}]_i$ responses to carbachol as well as thapsigargin. However, in a subsequent study on cells stably expressing TRP3, it was shown that the increased $[Ca^{2+}]_i$ signal with thapsigargin was due largely, if not entirely, to an increased basal permeability of the plasma mem-

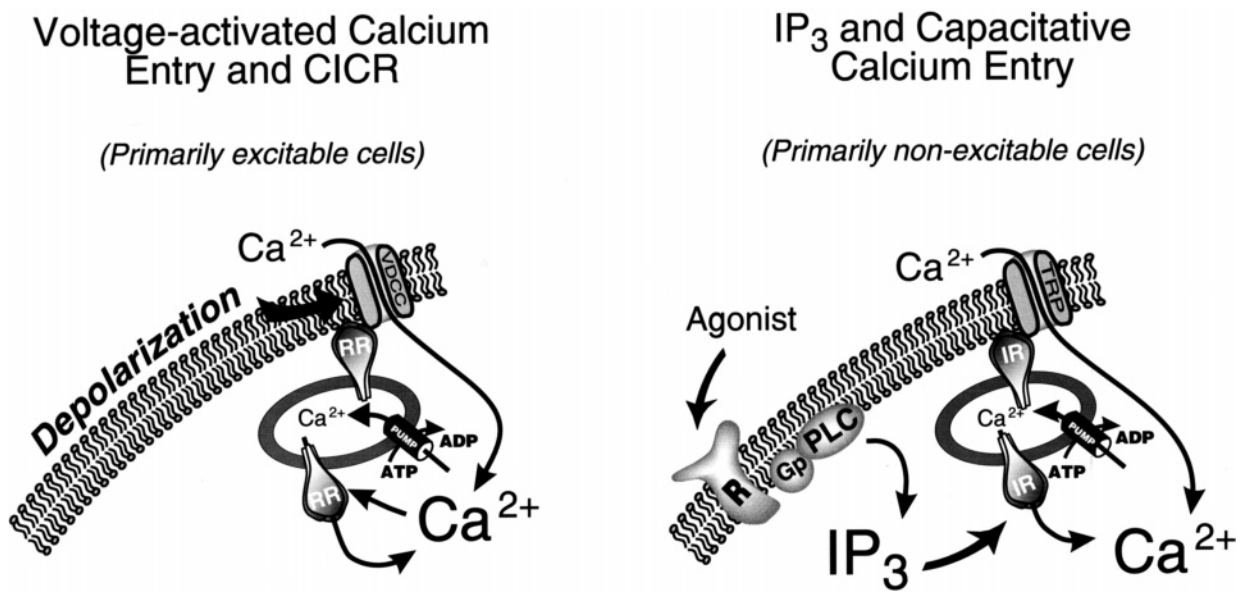

Figure 1. Endoplasmic reticulum: plasma membrane interactions in calcium signaling pathways. In electrically nonexcitable cells, (right) signaling is generally initiated when an agonist activates a surface membrane receptor (R) which, usually through a G protein (Gp), activates a phospholipase C (PLC) which degrades phosphatidylinositol 4,5-bisphosphate (PIP_2), releasing the soluble messenger inositol 1,4,5-trisphosphate (IP_3). IP_3 activates an IP_3 receptor (IR) and thus releases calcium from an intracellular organelle to the cytoplasm. The release of calcium from the organelle causes a signal to be generated which activates a plasma membrane calcium entry pathway (capacitative calcium entry). The favored model at present is conformational coupling whereby an IP_3 receptor interacts directly with plasma membrane channels, components of which are likely mammalian homologs of the *Drosophila* TRP protein. In electrically excitable cells, patterns of calcium signaling are somewhat more variable. Calcium may enter cells when voltage-dependent calcium channels (VDCC) are activated by the depolarization associated with action potentials. This calcium can cause further release of intracellularly stored calcium by activating a calcium-induced calcium release mechanism associated with the ryanodine receptor calcium channel (RR). Alternatively, as in the case of skeletal muscle, direct interaction of VDCC with RR can signal release of stored Ca^{2+}. Modified and redrawn from [166].

brane to Ca^{2+} [41]. On the basis of this and other results, it was concluded that TRP3 'forms a non-selective cation channel that opens after the activation of phospholipase C but not after store depletion' [41]. Zitt et al. [42] also concluded that TRP3 did not behave as a store-operated channel, and their data suggest it may be a Ca^{2+}-activated channel. However, subsequent studies on single-channel behavior of TRP3 were published utilizing another clone from the laboratory of reference [41], and these studies suggested that TRP3 was activated by store depletion and involved an interaction with IP_3 receptors [43]. For TRP1, publications from different laboratories report little or no effect of overexpression [38], constitutive activity not regulated by store depletion or phospholipase C [44], or activation by store depletion [45]. Expression of TRP4 and its structurally similar congener, TRP5, were reported to augment calcium-selective currents in response to calcium store depletion [46, 47]. Because of the clear association of these proteins with store-depletion-activated calcium currents, these investigators designated the genes CCE1 and CCE2 (for capacitative calcium entry) [47]. However, another laboratory cloned TRP5 and expressed it in HEK 293, the same cell type utilized in the previous studies, but found that the channel was activated directly by ATP receptors and not by store depletion [48]. This morass of conflicting results with overexpression is perhaps not too surprising, since other studies have reported unexpected behaviors of proteins when expressed in excessive quantities. A more convincing indictment of TRP proteins as mediators of capacitative calcium entry might come from specific perturbations of the native proteins, either pharmacologically or via molecular or genetic manipulations. There are few instances of such data, but the few that have been published are encouraging. Zhu et al. [38] reported that transfection of cells with a cocktail of antisense sequences against all six known (at the time) TRP proteins blocked capacitative calcium entry. Subsequently, this same group reported that antisense against TRP4 alone was sufficient to abrogate almost completely carbachol-induced calcium entry in mouse L cells [49]. Groschner et al. [50] reported that expression of an N-terminal fragment of TRP3 in endothelial cells blocked the activation of store-operated currents, presumably because the N-terminus of the protein is involved in channel assembly. Tomita et al. [51] reported partial inhibition of store-operated $[Ca^{2+}]_i$ signals in *Xenopus* oocytes following injection of antisense sequences directed against human TRP1. This result is somewhat surprising since the nucleotide sequence coding TRP proteins in *Xenopus* would be expected to be only somewhat similar to that of the corresponding mammalian gene. Other aspects of TRP proteins and their roles in mediating calcium entry have been discussed in recent reviews [28, 49, 52–55].

While evidence for the role of TRP proteins as capacitative calcium entry channels is steadily increasing, a more perplexing question involves the nature of the signal for activation of the plasma membrane channels. Two fundamentally different ideas have been suggested. Depletion of endoplasmic reticulum Ca^{2+} stores may trigger release or formation of a signaling substance that diffuses to the plasma membrane to activate the channels [27]. Alternatively, it has been suggested that proteins in the endoplasmic reticulum, specifically the IP_3 receptors, may directly interact with calcium channels in the plasma membrane via protein-protein interactions [56]. This idea was based on an analogy with the known interaction between ryanodine receptors and L-type calcium channels in skeletal muscle [30]. Although initially there was little direct evidence for this idea, recently strong evidence for interaction between IP_3 receptors and TRP channels has appeared. Kiselyov et al. [43] examined the behavior of TRP3 channels expressed in HEK293 cells. Single, nonselective 66 pS channels were activated by application of carbachol. On excision of the patches, activity was lost but could be restored by addition of IP_3. A similar observation had been made earlier by Vaca and Kunze with endogenous capacitative calcium entry channels in endothelial cells [57]. Kiselyov et al. found that with extensive washing of the excised patches, responsiveness to IP_3 was lost, and this could be restored by addition of IP_3 receptor, either in the natural environment of cerebellar microsomes or recombinant receptor in proteoliposomes. IP_3 receptor added without IP_3 present, however, was inactive [43]. Importantly, a study of endogenous single Ca^{2+} channels in A431 epidermal cells has demonstrated that the native store-operated channels are similarly regulated by IP_3 and the IP_3 receptor [58]. In a report examining interactions between TRP3 and IP_3 receptor constructs following their transient expression in HEK293 cells, it was demonstrated that the N-terminal IP_3 binding domain of the IP_3 receptor was sufficient to activate the TRP3 channels [59]. In the absence of a transmembrane domain to sense calcium store levels, the IP_3 binding domain constructs activated the channels independently of store depletion. Coexpression of the complete IP_3 receptor resulted in a complex that was sensitive to store depletion. These authors concluded that the association of TRP3 with an IP_3-liganded IP_3 receptor was obligatory and, in the absence of Ca^{2+} replete stores, sufficient for channel activation. Consistent with this interpretation, maneuvers that physically disrupt or interfere with interaction between plasma membrane and endoplasmic reticulum block the activation of capacitative calcium entry, without blocking the ability of IP_3 to release intracellular Ca^{2+} stores [60, 61]. Boulay et al. [62] in a recent report identified interacting sequences within the TRP3 and IP_3 receptor

molecules and demonstrated that expression of these peptide sequences in cells modulated capacitative calcium entry. Recently, Ma et al. [63] demonstrated that Ca^{2+} entry due to the overexpression of TRP3, or Ca^{2+} entry due to capacitative calcium entry, was completely blocked by a membrane-permeant IP_3 receptor antagonist, 2-aminoethoxydiphenyl borate. This provides strong evidence for the involvement of an IP_3 receptor in activation of both TRP3 and capacitative calcium entry channels [63, 64].

Regardless of the experimental protocol, it appears that regulation of TRP3 by IP_3 receptor requires the presence of IP_3. This implies that full activation of the channels requires that at least a subfraction of IP_3 receptors, those near the channels and involved in their activation, must be at or near saturation with IP_3. This may appear to present a problem, since it is known that drugs that deplete intracellular stores without activating phospholipase C can induce maximal activation of capacitative calcium entry [65] or the current associated with it, I_{crac} [66, 67]. However, it is know that in the *Drosophila* photoreceptor, phospholipase C exists in a scaffolded complex with TRP [68]. A similar arrangement in mammalian cells might bring the critical pool of TRP-coupled IP_3 receptors sufficiently close to phospholipase C such that they are IP_3-liganded even at resting phospholipase C activities [69].

While there is mounting evidence in favor of the idea that capacitative calcium entry channels are regulated by interaction with IP_3 receptors, this is certainly far from proven. In two independent studies, knockout of IP_3 receptors did not affect the ability of thapsigargin to activate calcium entry [70, 71]. In the former instance, an antisense strategy was used to knock down expression of the type 1 IP_3 receptor in Jurkat T lymphocytes. This resulted in essentially complete loss of response to phospholipase C (PLC)-linked agonists, whereas thapsigargin-induced entry was unaffected. However, the expression of the type 2 and type 3 receptors was not altered, and it is possible that one or both of these could have still functioned to regulate membrane-store-operated channels. In the latter study, the genes for all three IP_3 receptors were disrupted near the N-terminus of the protein. However, there is evidence that although this results in a protein incapable of gating Ca^{2+} in response to IP_3, the truncated IP_3 receptor can still couple to membrane TRP3 channels [43].

Evidence favoring a diffusible signal for capacitative calcium entry was presented in a study published by Parekh et al. [72]. These authors demonstrated that in *Xenopus* oocytes, the signaling from intracellular stores to plasma membrane channels could persist after physical disruption of connections between plasma membrane and intracellular organelles. However, a more recent report from Yao et al. [61] demonstrated that the current measured by Parekh et al. was unlikely to be a store-operated current and was more likely a calcium-activated current. These same investigators found that a number of manipulations that interfered with the secretory pathway blocked the activation of a store-operated current, and they proposed that a vesicle docking or fusion step might be involved in activation of entry. They also found that distention of the oocyte plasma membrane with a patch pipet prior to store depletion prevented activation of current under the pipet, but not elsewhere on the oocyte surface. This indicated that close proximity between the oocyte plasma membrane and underlying structures was required for coupling store depletion to channel activation [61].

Ribeiro et al. [73] found that severe cellular structural rearrangements associated with cytoskeletal disruption did not inhibit capacitative calcium entry (but see [74]). This observation was reproduced by Patterson et al. [60]. However, these same investigators reported that *stimulation* of actin polymerization by jasplakinolide or calyculin A blocked store-operated entry. This blockade was partially reversed by subsequent disruption of the actin filaments with cytochalasin D. The stimulation of actin polymerization resulted in a dense accumulation of actin filaments in the cell periphery and forced a retraction of the endoplasmic reticulum from the plasma membrane. Again, the conclusion was that close proximity between the plasma membrane and underlying structures, perhaps the endoplasmic reticulum, is required to activate the capacitative calcium entry channels. The findings of both Patterson et al. [60] and Yao et al. [61] are consistent with the idea that plasma membrane calcium channels, perhaps composed of TRP subunits, interact with underlying endoplasmic reticulum IP_3 receptors and that this interaction plays an obligatory role in their activation by depletion of intracellular Ca^{2+} stores.

Speculation about diffusible signals for entry have included suggestions of a role for cytochrome P450 metabolites [75–77], cyclic GMP [78–80] and an uncharacterized activity formed in store-depleted cells, termed CIF [81, 82]. In each case, conflicting data and conclusions have been subsequently published [28, 83–87]. In a recent report, a CIF activity from thapsigargin-treated Jurkat cells as well as a similar, and presumably identical activity from calcium pump-deficient yeast, was shown to activate Ca^{2+} influx in *Xenopus* oocytes and in Jurkat cells [82]. Confocal imaging of $[Ca^{2+}]_i$ in oocytes following injection of this material showed that the signals were restricted to the periphery of the cells, as expected for a substance acting specifically on the plasma membrane. Redistribution of intracellular organelles by centrifugation did not affect the progression of CIF-induced $[Ca^{2+}]_i$ signals across the oocyte, indicating that unlike signals initiated by IP_3,

the underlying organelles did not participate in the signaling process. When included in the patch pipet during whole-cell patch clamp of Jurkat cells, the extracts activated an inward current resembling the well-characterized I_{crac} [82], a Jurkat cell current believed to underlie capacitative calcium entry in this cell type [88]. These findings present perhaps the strongest evidence to date for a diffusible messenger mediating capacitative calcium entry. However, the properties of this entry were not identical to those of endogenous capacitative calcium entry in every respect. Notably, the Ca^{2+} signals were resistant to inhibition by lanthanum, whereas endogenous capacitative calcium entry is blocked by this trivalent cation. The nature and identity of the Ca^{2+}-signaling molecule present in CIF is as yet unknown, as well as the precise role it plays in physiological Ca^{2+} signaling pathways.

Endoplasmic reticulum calcium stores can regulate gene expression

If in fact a diffusible signal is generated by or released from the endoplasmic reticulum in response to calcium depletion, then it is possible that this signal may initiate or regulate other cellular responses. Until the identity of such a messenger is clearly established, this idea cannot be rigorously tested. However, there is a growing body of evidence that depletion of endoplasmic reticulum calcium stores can regulate processes other than calcium entry. In this section, we discuss the effects of calcium store depletion on gene expression, and in the subsequent section, effects on programmed cell death or apoptosis.

Indications that calcium stores might be involved in gene expression came shortly after the availability of thapsigargin as a specific tool for depleting intracellular stores. Thus, thapsigargin was shown to synergize with phorbol ester in activation of expression of chloramphenicol acetyltransferase from a construct containing artificial AP-1-like upstream sequences [89], and in activation of the early gene and protooncogene, c-fos [90, 91]. In the latter study, c-jun was activated by thapsigargin independently of protein kinase C activation. In both of these reports, however, the effects of thapsigargin were attributed to the rise in intracellular Ca^{2+} associated with release from the endoplasmic reticulum.

Some of the clearest examples of gene regulation associated with intracellular store depletion involve genes that code for proteins immediately impacted by or associated with endoplasmic reticulum calcium stores. The Lee laboratory has investigated the regulation of expression of specific heat-shock proteins, GRP78 and GRP94 [92]. These proteins are molecular chaperones which are induced by a number of different stresses in addition to heat shock, and are also calcium-binding proteins, BiP (GRP78) and endoplasmin (GRP94) [93]. Even before the availability of thapsigargin, Drummond et al. [94] demonstrated that expression of GRP78 and GRP94 could be upregulated by A23187, and that this induction was independent of the rise in cytoplasmic Ca^{2+}, resulting rather from the depletion of endoplasmic reticulum Ca^{2+}. The promoter for GRP78 contains a CCAAT motif that is activated by the human transcription factor, CBF [95]. In addition, the human nuclear factor YY1 can drive the GRP78 promoter under conditions of endoplasmic reticulum Ca^{2+} depletion [96]. However, neither CBF nor YY1 levels appear to be regulated by depletion of calcium stores. Activation by calcium store depletion of the expression of another major endoplasmic reticulum calcium-binding protein, calreticulin [97], also appears to involve CCAAT nucleotide sequences in the calreticulin promoter [97]. Finally, Kuo et al. [98] observed an induction by thapsigargin of expression of genes for both the plasma membrane and endoplasmic reticulum Ca^{2+} pumps, and as these responses were not diminished by loading the cells with the Ca^{2+} chelator, BAPTA, they concluded that it was depletion of Ca^{2+} stores that initiated this response.

As mentioned above, thapsigargin increases the expression of early genes, *fos* and *jun*, and thus might be expected to affect cellular growth and differentiation. In fact, the long-term effects of thapsigargin treatment on cellular functions are varied, including growth arrest in some instances [99, 100], and cell death through apoptosis in others [101–103]. In instances in which thapsigargin induces growth arrest, it is not clear whether this indicates a signal that directly results in growth arrest or simply a requirement for normally functioning Ca^{2+} pumps to signal cell division. Regardless, it seems paradoxical that thapsigargin does induce growth arrest but also induces the expression of early genes that are believed to be involved in signaling cellular growth and cell division. In the earlier studies, it was not clear whether the effects of thapsigargin resulted from a rise in $[Ca^{2+}]_i$ or depletion of Ca^{2+} stores. A recent paper from Qi et al. [104] suggests that c-fos induction by thapsigargin is due to an increase in Ca^{2+} in the cytoplasm. Thus, it is possible that the stimulatory effects of thapsigargin involve cytoplasmic Ca^{2+}, presumably through activation of CRE [105, 106], whereas store depletion, or the lack of functional endoplasmic reticulum Ca^{2+} pumps, is responsible for growth arrest.

An interesting interplay between genes involved in cell cycle regulation was revealed in a study in which vascular smooth muscle cells were stably transfected with a dominant negative against c-myb, a protooncogene required for cell cycle progression [107]. The result of diminished c-myb activity was a significant decrease in cell proliferation as well as a reduction in the size of the

thapsigargin-sensitive Ca^{2+} pool [107]; however, the reduction in Ca^{2+} storage was clearly not a result of growth arrest. Thus, the status of Ca^{2+} pools can influence the expression of genes involved in regulating cell growth, and the activity of growth-regulating genes in turn can influence the size and status of endoplasmic reticulum Ca^{2+} pools.

In recent years, other instances of either activation [108] or inhibition [109] of gene expression clearly linked to depletion of endoplasmic reticulum Ca^{2+} have been reported. These findings raise the general question of the role of intracellular Ca^{2+} storage in the response to other Ca^{2+}-mobilizing stimuli, whether pharmacological (ionophores, SERCA inhibitors) or physiological (phospholipase C-linked agonists).

Endoplasmic reticulum calcium stores can regulate apoptosis

It has long been known that artificial mobilization of cellular Ca^{2+}, with Ca^{2+} ionophores, can serve as a full and sufficient stimulus for programmed cell death, or apoptosis [110, 111]. More recently, a number of laboratories have demonstrated that the SERCA inhibitor thapsigargin is a potent inducer of apoptosis [101–103, 112, 113]. However, only recently have investigations addressed the issue of the relative roles of cytoplasmic and endoplasmic reticulum stored Ca^{2+}.

Glucocorticoid-induced apoptosis of lymphoid cells is a widely studied model of cell death. In a study of glucocorticoid-induced apoptosis Kaiser and Edelman [114] identified a Ca^{2+} influx which they concluded was associated with glucocorticoid-induced lymphoid cell death. Investigators have shown that chelation of Ca^{2+} by intracellular chelators, extracellular EGTA or overexpression of the calcium-binding protein, calbindin, inhibits apoptosis due to glucocorticoids and other agents [115–119]. Calcium channel blockers also prevent apoptosis in regressing prostate [120]. The functional activity of Ca^{2+} is often mediated through binding to calmodulin [121–123], and calmodulin antagonists have been reported to disrupt apoptosis in a variety of systems [115, 124]. The oncogene *Bcl-2*, the product of which is known to inhibit apoptosis in several model systems, has also been suggested to regulate intracellular Ca^{2+} compartmentalization [125, 126]. Lam et al. [126, 127] demonstrated that dexamethasone elevated $[Ca^{2+}]_i$ and reduced stored Ca^{2+} in a lymphocytic cell line and concluded that glucocorticoids may act in a thapsigargin-like manner. Together these data suggest a central role for calcium in apoptosis in response to glucocorticoids and other agents.

The endonuclease responsible for internucleosomal cleavage of DNA appears to be dependent on Ca^{2+}, suggesting that increased Ca^{2+} may be necessary to allow (or activate) nuclease activity. Cleavage of chromatin into large DNA fragments (50 Kb) has also been suggested to require Ca^{2+} [128], although other studies [129] disagree. Other types of Ca^{2+}-dependent enzymes (such proteases and lipases [130]) have also been implicated in apoptosis. Whether Ca^{2+} plays a direct role (as activator) or a passive role (as cofactor) with these enzymes has not been determined. However, Lam et al. [126] point out that rather than an elevation of cytoplasmic Ca^{2+}, depletion of Ca^{2+} stores is also a potentially important factor in the action of thapsigargin, as lowering of endoplasmic Ca^{2+} content can have a number of deleterious effects on cells, for example by interfering with protein synthesis [131] (see also [113]).

Bian et al. [103] examined the role of Ca^{2+} signalling in apoptosis induced in S49 cells (a lymphocytic line) by the Ca^{2+}-ATPase inhibitors thapsigargin and cyclopiazonic acid, and by the synthetic glucocorticoid dexamethasone. These investigators also investigated the effects of overexpression of the antiapoptotic oncogene Bcl-2. In support of the idea that depletion of stored Ca^{2+} may signal apoptosis, Bian et al. [103] found that removal of extracellular Ca^{2+} augmented rather than inhibited apoptosis due to thapsigargin. Overexpression of the apoptosis suppressor Bcl-2 inhibited apoptosis due to thapsigargin but did not affect thapsigargin-induced Ca^{2+} signaling. Dexamethasone induced apoptosis, diminished the size of the endoplasmic reticulum Ca^{2+} pool and caused a small elevation of intracellular Ca^{2+}, results similar to those originally reported by Lam et al. [126, 127, 131]. However, this elevation was not due to Ca^{2+} influx, because the increase was similar in the presence or absence of Ca^{2+} in the medium. Furthermore, in contrast to the results with thapsigargin, apoptosis due to dexamethasone was unchanged in a Ca^{2+}-free medium. These findings indicate that changes in Ca^{2+} handling appear to play a lesser role than previously thought in the actions of Bcl-2 and glucocorticoids. They also indicate that depletion of intracellular stores can provide a potent stimulus for apoptosis, independent of the activation of Ca^{2+} entry. This may mean that the signaling pathway activated by store depletion also triggers apoptosis. This could even involve the same messenger from the endoplasmic reticulum, although the available data do not address this possibility. Alternatively, it is possible that the inhibition of protein synthesis or loss of chaperone function resulting from severe depletion of intracellular stores is responsible. Previous studies have shown that inhibition of protein synthesis by cycloheximide can initiate apoptosis [132, 133]. In a recent report, Pinton et al. [134] reported that partial reduction of endoplasmic reticulum calcium stores actually protected against apoptosis.

There is evidence for involvement of the IP_3 receptor in lymphocyte apoptosis. Khan et al. [135] found that lymphocytes undergoing apoptosis increased their expression of the type 3 IP_3 receptor, and their data indicated that this was primarily in the plasma membrane. Although the presence of IP_3 receptors in the plasma membrane is controversial, there is evidence that some type 3 IP_3 receptors may be located in the plasma membrane where they are regulated by intracellular Ca^{2+} stores as well as by IP_3 directly [136]. Type 1 receptor was not increased, and antisense against type 3, but not antisense against type 1 receptor blocked dexamethasone-induced apoptosis. In striking contrast, Jayaraman and Marks [137] reported that antisense reduction in type 1 IP_3 receptor rendered Jurkat T lymphocytes resistant to apoptosis in response to dexamethasone, T-cell-receptor stimulation, ionizing radiation and Fas receptor activation. In an earlier report, this same group reported that the type 1 IP_3 receptor-deficient lymphocytes did not respond to T-cell-receptor activation but were still capable of responding to thapsigargin with both Ca^{2+} release and Ca^{2+} entry [70]. Jayaraman and Marks also noted that in Jurkat cells, the induction of apoptosis was not reduced in the absence of external Ca^{2+} and concluded that the IP_3 receptor was needed to mediate release of Ca^{2+} from the endoplasmic reticulum [137]. The reason for the difference in the results from these two laboratories is not readily apparent. However, different cell lines were used in the two studies, and this could be significant (WEHI-231 B cells and S-49 T cells in the study by Kahn et al. [135], and Jurkat T cells in the study by Jayaraman and Marks [137]).

Bcl-2 and Bcl-xl are protooncogenes which, when expressed in excess, appear to suppress apoptosis [138, 139]. Other members of this family of proteins with similar structures facilitate apoptosis, for example, Bax [140]. These proteins appear to be primarily distributed to mitochondria [138], where they may regulate cytochrome C release, a key step in the apoptotic pathway [141]. These proteins also structurally resemble ion channels, and some have been shown to function as ion channels in bilayer assays [142, 143]. However, since different members of this protein family can produce essentially opposite effects on the apoptotic pathway, it is not clear whether this channel activity reflects a true cellular function.

Despite the observed mitochondrial localization of Bcl-2, its overexpression clearly modulates endoplasmic calcium storage, especially when apoptosis is stimulated [103, 144]. As mentioned above, Bcl-2 protects cells from apoptosis caused by a number of agents, including glucocorticoids and thapsigargin. He et al. [144] found that Bcl-2 diminished the rate of Ca^{2+} loss from the endoplasmic reticulum due to either thapsigargin treatment, or low extracellular Ca^{2+}. Bian et al. [103] reported that dexamethasone treatment of S49 lymphocytes diminished endoplasmic reticulum Ca^{2+} storage, and this effect was antagonized by Bcl-2. However, in contrast to the findings of He et al., Bian et al. observed no effect of Bcl-2 on thapsigargin-induced $[Ca^{2+}]_i$ signals. This difference probably reflects the fact that Bian et al. utilized a supramaximal concentration of thapsigargin (2 μM), whereas He et al. used a significantly lower concentration (100 nM). Kuo et al. [145] demonstrated that Bcl-2 overexpression caused an upregulation of SERCA expression [both messenger RNA (mRNA) and protein] and found that Bcl-2 could be co-immunoprecipitated with SERCA. With an increased number of SERCA pumps, one might expect resistance to submaximal, but not to supramaximal concentrations of thapsigargin. In aggregate, these findings strongly suggest that Bcl-2 protects cells from apoptosis at least in part by augmenting Ca^{2+} pumping into the endoplasmic reticulum, thus resisting the depletion of Ca^{2+}, which can apparently play a significant role in the signaling pathway leading to cellular apoptosis.

Regulation of endoplasmic reticulum structure and function by signaling pathways

Regulation by calcium

The first studies suggesting a role for $[Ca^{2+}]_i$ in the modulation of endoplasmic reticulum structure were performed in sea urchin eggs. Terasaki and Jaffe utilized fluorescent lipophilic dicarbocyanine dyes (referred to as DiI) which, upon injection of a saturated solution in soybean oil into the cytoplasm, spread from the oil drop throughout the endoplasmic reticulum network [146–148]. Through the use of confocal microscopy, this method enabled direct imaging of the dynamic nature of this organelle under a variety of conditions, such as during meiotic maturation and fertilization [146, 148, 149].

In sea urchin and starfish eggs, fertilization causes IP_3 generation and release of sequestered calcium, leading to a spreading wave of $[Ca^{2+}]_i$ mobilization. When this wave of $[Ca^{2+}]_i$ mobilization passes through the confocal field of view, it is associated with a transient fragmentation of the endoplasmic reticulum, seen with DiI. It was suggested that this temporary fragmentation may be a fundamental event of Ca^{2+} release at fertilization [146, 148, 149]. Recently, starfish eggs expressing green fluorescent protein (GFP) targeted to the lumen of the endoplasmic reticulum were used to address the issue of loss of endoplasmic reticulum continuity during fertilization. By applying fluorescence recovery after photobleaching (FRAP) technology it was shown that the

time required for FRAP was much longer for oocytes at 1 min postfertilization than in unfertilized eggs or in 20 min postfertilized eggs [150]. These findings were consistent with the previously published DiI data, providing further evidence for a temporary loss of endoplasmic reticulum continuity at fertilization. However, an important question still remained: Is endoplasmic reticulum fragmentation during fertilization a result of the loss of sequestered calcium or the rise in $[Ca^{2+}]_i$? This issue was addressed in a report by Subramanian and Meyer [151] who showed that in rat basophilic leukemia (RBL) cells expressing GFP in the endoplasmic reticulum, high $[Ca^{2+}]_i$, but not Ca^{2+} pool depletion per se, was responsible for vesiculating that organelle; furthermore, the effect of high $[Ca^{2+}]_i$ on endoplasmic reticulum continuity appeared to be specific for the endoplasmic reticulum, since the integrity of the nuclear envelope, an extension of the endoplasmic reticulum, was maintained under high $[Ca^{2+}]_i$ conditions.

The issue of endoplasmic reticulum fragmentation by increased $[Ca^{2+}]_i$ levels has been investigated in human embryonic kidney (HEK 293) cells stably transfected with GFP targeted to the endoplasmic reticulum [152]. Following Ca^{2+} store-depleting protocols (e.g. thapsigargin or ionomycin in Ca^{2+}-free medium or in media with different Ca^{2+} concentrations), the endoplasmic reticulum network appeared to fragment (as revealed by punctate GFP fluorescence) only when $[Ca^{2+}]_i$ was sustained in the μM range by the calcium ionophore ionomycin (fig. 2). The effect of high $[Ca^{2+}]_i$ on GFP fluorescence was not due to changes in the distribution or fluorescent properties of GFP, since no similar formation of punctate structures was seen in cells expressing cytoplasmic GFP [152]. The punctate nature of the endoplasmic reticulum was not due to nonspecific fragmentation and swelling of the endoplasmic reticulum as revealed by fine structural analysis by electron microscopy [152]. In control cells, the endoplasmic reticulum appeared as parallel membranes decorated with ribosomes. Under high $[Ca^{2+}]_i$ conditions clearly distinct and, in some cases, circular and in other cases unusual, elaborately branching endoplasmic reticulum structures were seen. These are presumed to be the result of membrane folding and fusion induced by the elevated $[Ca^{2+}]_i$. These branched clusters of endoplasmic reticulum retained their classical double-membrane configuration and apparently represent the fluorescent spots observed with the endoplasmic-reticulum-directed GFP under high and sustained $[Ca^{2+}]_i$. These findings indicate that endoplasmic reticulum structure can undergo profound changes, resulting in loss of its continuous, networklike structure when $[Ca^{2+}]_i$ levels are elevated and sustained. The implication of these findings for the function of this organelle in $[Ca^{2+}]_i$ signaling will be discussed below.

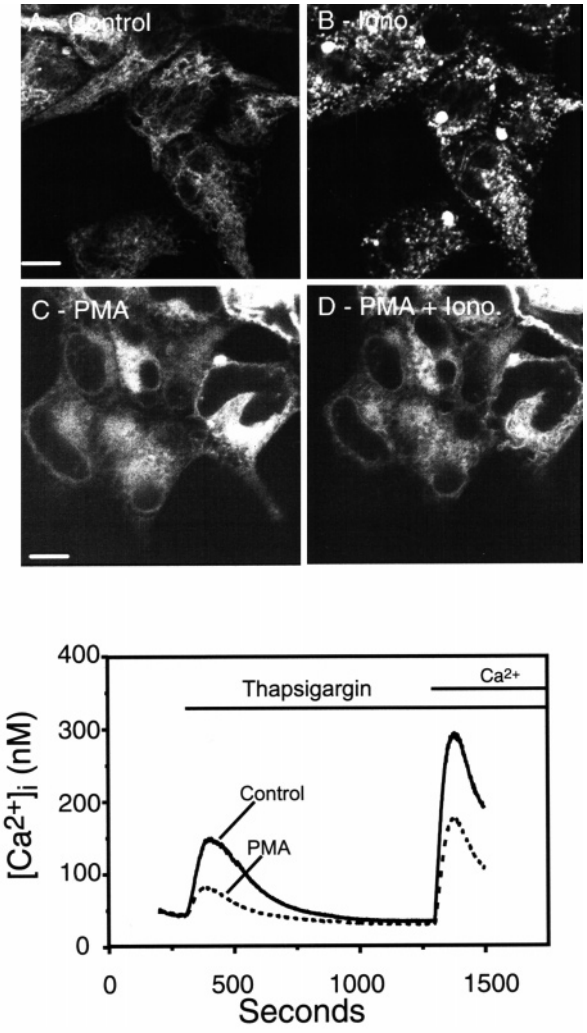

Figure 2. Protein kinase C and Ca^{2+} effects on endoplasmic reticulum structure and function. (*Top*) PMA pretreatment prevents ionomycin-induced endoplasmic reticular alterations. HEK cells expressing a GFP construct targeted to the endoplasmic reticulum were treated with the vehicle used for dissolving PMA, DMSO (0.1%) (control, A and B) or 1.6 μM PMA (C and D) for 60 min at 37 °C. The endoplasmic reticulum morphology was subsequently visualized by confocal microscopy. A and C: endoplasmic reticulum fluorescence before treatment with ionomycin; B and D: endoplasmic reticulum fluorescence 10 min after addition of 10 μM ionomycin in 1.8 mM Ca^{2+}-containing buffer. Ionomycin-induced alterations in endoplasmic reticulum structure are prevented in PMA-treated cells. Bar, 10 μm. (*Bottom*) PMA reduces the apparent size of the thapsigargin-sensitive Ca^{2+} pool and decreases capacitative Ca^{2+} entry. HEK cells were treated with DMSO (control, solid line), or 1.6 μM PMA (dotted line) and intracellular Ca^{2+} determined by fura-2 fluorescence. The concentration of thapsigargin was 2 μM and was present during the interval indicated by the horizontal line. In this experiment, the cells were initially kept in a media lacking calcium, and calcium was restored during the interval as indicated. The thapsigargin-releasable Ca^{2+} pool (response on first addition of thapsigargin) as well as capacitative Ca^{2+} influx (the response on readdition of Ca^{2+}) are decreased in PMA-treated cells compared to controls. Redrawn and modified from data originally presented in [152].

Regulation by protein kinase C

It is well established that protein kinase C (PKC) regulates many aspects of Ca^{2+} signaling. These include inhibition of IP_3 production [153–155], thereby inhibiting hormone-dependent Ca^{2+} release, and facilitation [156, 157] or inhibition [156, 158–161] of capacitative Ca^{2+} entry. PKC activation with phorbol 12-myristate 13-acetate (PMA) also causes a marked reduction in the Ca^{2+} storage capacity of the endoplasmic reticulum in NIH 3T3 cells [162]. Interestingly, capacitative Ca^{2+} entry was not activated by this depletion, and subsequent depletion of the remaining stores by thapsigargin resulted in normal activation of capacitative calcium entry. These findings suggested that activation of PKC can alter the Ca^{2+}-storing function of the endoplasmic reticulum without altering its ability to control capacitative Ca^{2+} entry. The effect of PKC activation on diminishing the capacity of Ca^{2+} stores was also observed in an epidermal cell line (A431) and in freshly isolated lacrimal cells [162], as well as in glioma C6 cells [163] and platelets [164], indicating that this is a general phenomenon. The mechanism responsible for the action of PKC on Ca^{2+} storage is not known, but there appear to be little or no changes in the basic Ca^{2+} transport systems underlying endoplasmic reticulum Ca^{2+} storage (e.g. the endoplasmic reticulum Ca^{2+}-ATPase, the endoplasmic reticulum leak channel or the plasma membrane Ca^{2+}-ATPase) [162]. Interestingly, in NIH 3T3 cells overexpressing PKC isoforms, treatment with PMA caused activated PKC-α to concentrate in punctate regions in the endoplasmic reticulum [165]; therefore, it is reasonable to speculate that activation of this PKC isoform and its association with components of the endoplasmic reticulum could be related to the loss of sequestered Ca^{2+}.

A recent study investigated the effect of PKC activation by PMA on endoplasmic reticulum function and structure in HEK 293 cells. Endoplasmic reticulum function as a Ca^{2+} store was addressed by measuring Ca^{2+} store capacity and Ca^{2+} entry, whereas the effect of PMA on endoplasmic reticulum structure was investigated in cells stably transfected with GFP targeted to the endoplasmic reticulum [152]. As in earlier work with NIH 3T3 cells [162], PMA induced a loss of sequestered Ca^{2+}, but in HEK cells PMA also inhibited capacitative Ca^{2+} entry [152] (fig. 2). When endoplasmic reticulum structure was examined in cells transfected with endoplasmic-reticulum-targeted GFP, depletion of Ca^{2+} stores by PMA or the SERCA inhibitor thapsigargin induced little or no change in endoplasmic reticulum structure. However, pretreatment with PMA completely prevented the effect of elevated $[Ca^{2+}]_i$ in inducing endoplasmic reticulum fragmentation [152] (fig. 2). This finding, especially when considered with the precise morphological nature of the Ca^{2+}-induced rearrangements, indicates that the effects of high $[Ca^{2+}]_i$ in modifying endoplasmic reticulum structure is not a nonspecific, toxic effect of elevated $[Ca^{2+}]_i$. Rather, these responses would seem to reflect an experimentally exaggerated manifestation of some as yet uncharacterized physiological mechanism through which the Ca^{2+} and PKC pathways influence and regulate endoplasmic reticulum structure. It may be inferred from the ability of PKC to protect the endoplasmic reticulum from fragmentation that the alterations of endoplasmic reticulum structure seen under high $[Ca^{2+}]_i$ conditions involve the Ca^{2+} activation of proteins that can be modulated by PKC-mediated phosphorylation. These changes may occur in more subtle and directed ways under physiological conditions. For example, changes in the endoplasmic reticulum may occur transiently during the brief but large rises in $[Ca^{2+}]_i$ associated with $[Ca^{2+}]_i$ spikes and oscillations [20]. The transient loss in endoplasmic reticulum continuity upon fertilization of starfish oocytes, an event which correlates with Ca^{2+} mobilization, may represent one documented example of such a response occurring during a physiological process.

Conclusions

The purpose of this review has been to summarize current advances in our knowledge of the Ca^{2+}-signaling functions of endoplasmic reticulum. This includes the 'classical' endoplasmic reticulum functions involving release of activator Ca^{2+}, and less well understood functions involving signaling from the depletion of endoplasmic reticulum Ca^{2+} stores. The release of Ca^{2+} to the cytoplasm as well as Ca^{2+} store depletion appear to be involved in regulation of long-term, fundamental cellular processes such as the control of gene expression, and cellular growth and cellular apoptosis.

Both the Ca^{2+} and PKC pathways have potentially profound but poorly understood effects on the structure and signaling functions of the endoplasmic reticulum. There are also intriguing but at present mysterious modes of interaction of these basic signaling pathways. Recent work has revealed novel actions of the PKC pathway, including a reduction of intracellular Ca^{2+} storage capacity, activation or inhibition of capacitative Ca^{2+} entry, and protection of the endoplasmic reticulum against the effects of high $[Ca^{2+}]_i$. The emerging view is one of a complex network of signaling pathways through which the endoplasmic reticulum and the Ca^{2+} and PKC pathways interact at various levels to regulate cellular structure and function.

1 Rasmussen H. (1985) Calcium ion. A synarchic and mercurial but minatory messenger. In: Calcium in Biological Sys-

tems, pp. 13–22, Rubin R. P., Weiss G. B. and Putney J. W. Jr (eds), Plenum Press, New York
2 Ringer S. (1883) A further contribution regarding the influence of the different constituents of the blood on the contraction of the heart. J. Physiol. (Lond.) **4:** 29–42
3 Bianchi C. P. (1968). Cell Calcium. Butterworths, London
4 Ebashi S. (1991) Excitation-contraction coupling and the mechanism of muscle contraction. Ann. Rev. Physiol. **53:** 1–16
5 Putney J. W. Jr, Poggioli J. and Weiss S. J. (1981) Receptor regulation of calcium release and calcium permeability in parotid gland cells. Phil. Trans. R. Soc. Lond. B **296:** 37–45
6 Burgess G. M., McKinney J. S., Fabiato A., Leslie B. A. and Putney J. W. Jr. (1983) Calcium pools in saponin-permeabilized guinea-pig hepatocytes. J. Biol. Chem. **258:** 15336–15345
7 Meldolesi J., Volpe P. and Pozzan T. (1988) The intracellular distribution of calcium. Trends Neurosci. **11:** 449–452
8 Pozzan T., Rizzuto R., Volpe P. and Meldolesi J. (1994) Molecular and cellular physiology of intracellular calcium stores. Physiol. Rev. **74:** 595–636
9 Rossier M. F. and Putney J. W. Jr (1991) The identity of the calcium storing inositol 1,4,5-trisphosphate-sensitive organelle in non-muscle cells: calciosome, endoplasmic reticulum,... or both? Trends Neurosci. **14:** 310–314
10 Meldolesi J. and Pozzan T. (1998) The endoplasmic reticulum Ca2+ store: a view from the lumen. Trends Biochem. Sci. **23:** 10–14
11 Schneider M. F. (1994) Control of calcium release in functioning muscle fibers. Ann. Rev. Physiol. **56:** 463–484
12 Inui M., Saito A. and Fleischer S. (1987) Purification of the ryanodine receptor and identity with feet structures of junctional terminal cisternae of sarcoplasmic reticulum from fast skeletal muscle. J. Biol. Chem. **262:** 1740–1747
13 Imagawa T., Smith J. S., Coronado R. and Campbell K. P. (1987) Purified ryanodine receptor from skeletal muscle sarcoplasmic reticulum is the Ca^{2+}-permeable pore of the calcium release channel. J. Biol. Chem. **262:** 16636–16643
14 Berridge M. J. (1993) Inositol trisphosphate and calcium signalling. Nature **361:** 315–325
15 Taylor C. W. and Marshall C. B. (1992) Calcium and inositol 1,4,5-trisphosphate receptors: a complex relationship. Trends Biochem. Sci. **17:** 403–407
16 Ehrlich B. E., Kaftan E., Bezprozvannaya S. and Bezprozvanny I. (1994) The pharmacology of intracellular Ca^{2+}-release channels. Trends Pharmacol. Sci. **15:** 145–149
17 Lee H. C. (1997) Mechanisms of calcium signaling by cyclic ADP-ribose and NAADP. Physiol. Rev. **77:** 1133–1164
18 Galione A. (1994) Cyclic ADP-ribose, the ADP-ribosyl cyclase pathway and calcium signalling. Mol. Cell. Endocrinol. **98:** 125–131
19 Guse A. H., da Silva C. P., Berg I., Skapenko A. L., Weber K., Heyer P. et al. (1999) Regulation of calcium signalling in T lymphocytes by the second messenger cyclic ADP-ribose. Nature **398:** 70–73
20 Thomas A. P., Bird G. St J., Hajnóczky G., Robb-Gaspers L. D. and Putney J. W. Jr (1996) Spatial and temporal aspects of cellular calcium signalling. FASEB J. **10:** 1505–1517
21 Bootman M. D., Berridge M. J. and Lipp P. (1997) Cooking with calcium: the recipes for composing global signals from elementary events. Cell **91:** 367–373
22 Tse A., Tse F. W., Almers W. and Hille B. (1993) Rhythmic exocytosis stimulated by GnRH-induced calcium oscillations in rat gonadotropes. Science **260:** 82–84
23 Maruyama Y., Inooka G., Li Y. X., Miyashita Y. and Kasai H. (1993) Agonist-induced localized Ca^{2+} spikes directly triggering exocytotic secretion in exocrine pancreas. EMBO J. **12:** 3017–3022
24 Dolmetsch R. E., Xu K. L. and Lewis R. S. (1998) Calcium oscillations increase the efficiency and specificity of gene expression. Nature **392:** 933–936
25 Li W.-H., Llopis J., Whitney M., Zlokarnik G. and Tsien R. Y. (1998) Cell-permeant caged InsP3 ester shows that Ca2+ spike frequency can optimize gene expression. Nature **392:** 936–941
26 Putney J. W. Jr (1986) A model for receptor-regulated calcium entry. Cell Calcium **7:** 1–12
27 Putney J. W. Jr (1990) Capacitative calcium entry revisited. Cell Calcium **11:** 611–624
28 Putney, J. W. Jr. (1997) Capacitative Calcium Entry. Landes Biomedical Publishing, Austin, TX
29 Clapham D. E. (1995) Intracellular calcium. Replenishing the stores. Nature **375:** 634–635
30 Berridge M. J. (1995) Capacitative calcium entry. Biochem. J. **312:** 1–11
31 Hardie R. C. and Minke B. (1993) Novel Ca^{2+} channels underlying transduction in *Drosophila* photoreceptors: implications for phosphoinositide-mediated Ca^{2+} mobilization. Trends Neurosci. **16:** 371–376
32 Ranganathan R., Bacskai B. J., Tsien R. Y. and Zuker C. S. (1994) Cytosolic calcium transients: spatial localization and role in *Drosophila* photoreceptor cell function. Neuron **13:** 837–848
33 Vaca L., Sinkins W. G., Hu Y., Kunze D. L. and Schilling W. P. (1994) Activation of recombinant *trp* by thapsigargin in Sf9 insect cells. Am. J. Physiol. **267:** C1501–C1505
34 Petersen C. C. H., Berridge M. J., Borgese M. F. and Bennett D. L. (1995) Putative capacitative calcium entry channels: expression of *Drosophila trp* and evidence for the existence of vertebrate homologues. Biochem. J. **311:** 41–44
35 Gillo B., Chorna I., Cohen H., Cook B., Manistersky I., Chorev M. et al. (1996) Coexpression of *Drosophila* TRP and TRP-like proteins in *Xenopus* oocytes reconstitutes capacitative Ca^{2+} entry. Proc. Natl. Acad. Sci. USA **93:** 14146–14151
36 Zhu X., Chu P. B., Peyton M. and Birnbaumer L. (1995) Molecular cloning of a widely expressed human homologue for the *Drosophila trp* gene. FEBS Lett. **373:** 193–198
37 Wes P. D., Chevesich J., Jeromin A., Rosenberg C., Stetten G. and Montell C. (1995) TRPC1, a human homolog of a *Drosophila* store-operated channel. Proc. Natl. Acad. Sci. USA **92:** 9652–9656
38 Zhu X., Jiang M., Peyton M., Boulay G., Hurst R., Stefani E. et al. (1996) *trp*, a novel mammalian gene family essential for agonist-activated capacitative Ca^{2+} entry. Cell **85:** 661–671
39 Okada T., Inoue R., Yamazaki K., Maeda A., Kurosaki T., Yamakuni T. et al. (1999) Molecular and functional characterization of a novel mouse transient receptor potential protein homologue TRP7. Ca^{2+}-permeable cation channel that is constitutively activated and enhanced by stimulation of G protein-coupled receptor. J. Biol. Chem. **274:** 27359–27370
40 Vannier B., Peyton M., Boulay G., Brown D., Qin N., Jiang M. et al. (1999) Mouse trp2, the homologue of the human trpc2 pseudogene, encodes mTrp2, a store depletion-activated capacitative Ca^{2+} channel. Proc. Natl. Acad. Sci. USA **96:** 2060–2064
41 Zhu X., Jiang M. and Birnbaumer L. (1998) Receptor-activated Ca^{2+} influx via human Trp3 stably expressed in human embryonic kidney (HEK)293 cells. J. Biol. Chem. **273:** 133–142
42 Zitt C., Obukhov A. G., Strübing C., Zobel A., Kalkbrenner F., Lückhoff A. et al. (1997) Expression of TRPC3 in Chinese hamster ovary cells results in calcium-activated cation currents not related to store depletion. J. Cell Biol. **138:** 1333–1341
43 Kiselyov K., Xu X., Mozhayeva G., Kuo T., Pessah I., Mignery G. et al. (1998) Functional interaction between $InsP_3$ receptors and store-operated Htrp3 channels. Nature **396:** 478–482
44 Sinkins W. G., Estacion M. and Schilling W. P. (1998) Functional expression of TrpC1: a human homologue of the *Drosophila* Trp channel. Biochem. J. **331:** 331–339

45 Zitt C., Zobel A., Obukhov A. G., Harteneck C., Kalkbrenner F., Lückhoff A. et al. (1996) Cloning and functional expression of a human Ca^{2+}-permeable cation channel activated by calcium store depletion. Neuron **16:** 1189–1196

46 Philipp S., Cavalié A., Freichel M., Wissenbach U., Zimmer S., Trost C. et al. (1996) A mammalian capacitative calcium entry channel homologous to *Drosophila* TRP and TRPL. EMBO J. **15:** 6166–6171

47 Philipp S., Hambrecht J., Braslavski L., Schroth G., Freichel M. et al. (1998) A novel capacitative calcium entry channel expressed in excitable cells. EMBO J. **17:** 4274–4282

48 Okada T., Shimizu S., Wakamori M., Maeda A., Kurosaki T., Takada N. et al. (1998) Molecular cloning and functional characterization of a novel receptor-activated TRP Ca^{2+} channel from mouse brain. J. Biol. Chem. **273:** 10278–10279

49 Birnbaumer L., Zhu X., Jiang M., Boulay G., Peyton M., Vannier B. et al. (1996) On the molecular basis and regulation of cellular capacitative calcium entry: roles for Trp proteins. Proc. Natl. Acad. Sci. USA **93:** 15195–15202

50 Groschner K., Hingel S., Lintschinger B., Balzer M., Romanin C., Zhu X. et al. (1998) Trp proteins form store-operated cation channels in human vascular endothelial cells. FEBS Lett. **437:** 101–106

51 Tomita Y., Kaneko S., Funayama M., Kondo H., Satoh M. and Akaike A. (1998) Intracellular Ca^{2+} store-operated influx of Ca^{2+} through TRP-R, a rat homolog of TRP, expressed in *Xenopus* oocytes. Neurosci. Letters **248:** 195–198

52 Friel D. D. (1996) TRP: its role in phototransduction and store-operated Ca^{2+} entry. Cell **85:** 617–619

53 Hardie R. C. (1996) Calcium signalling: setting store by calcium channels. Curr. Biol. **6:** 1371–1373

54 Montell C. (1997) New light on TRP and TRPL. Mol. Pharmacol. **52:** 755–763

55 Putney J. W. Jr and McKay R. R. (1999) Capacitative calcium entry channels. Bioessays **21:** 38–46

56 Irvine R. F. (1990) 'Quantal' Ca^{2+} release and the control of Ca^{2+} entry by inositol phosphates-a possible mechanism. FEBS Lett. **263:** 5–9

57 Vaca L. and Kunze D. L. (1994) Depletion of intracellular Ca^{2+} stores activates a Ca^{2+}-selective channel in vascular endothelium. Am. J. Physiol. **267:** C920–C925

58 Zubov A. I., Kaznacheeva E. V., Alexeeno V. A., Kiselyov K., Muallem S. and Mozhayeva G. (1999) Regulation of the miniature plasma membrane Ca^{2+} channel I_{min} by IP_3 receptors. J. Biol. Chem. **274:** 25983–25985

59 Kiselyov K., Mignery G. A., Zhu M. X. and Muallem S. (1999) The N-terminal domain of the IP_3 receptor gates store-operated hTrp3 channels. Mol. Cell **4:** 423–429

60 Patterson R. L., van Rossum D. B. and Gill D. L. (1999) Store-operated Ca^{2+} entry: evidence for a secretion-like coupling model. Cell **98:** 487–499

61 Yao Y., Ferrer-Montiel A. V., Montal M. and Tsien R. Y. (1999) Activation of store-operated Ca^{2+} current in *Xenopus* oocytes requires SNAP-25 but not a diffusible messenger. Cell **98:** 475–485

62 Boulay G., Brown D. M., Qin N., Jiang M., Dietrich A., Zhu M. X. et al. (1999) Modulation of Ca^{2+} entry by TRP-binding polypeptides of the inositol 1,4,5-trisphosphate receptor (IP3R). Evidence for roles of TRP and the IP3R in store depletion-activated Ca^{2+} entry. Proc. Nat. Acad. Sci. USA **96:** 14955–17960

63 Ma H.-T., Patterson R. L., van Rossum D. B., Birnbaumer L., Mikoshiba K. and Gill D. L. (2000) Requirement of the inositol trisphosphate receptor for activation of store-operated Ca^{2+} channels. Science **287:** 1647–1651

64 Berridge M. J., Lipp P. and Bootman M. (2000) The calcium entry pas de deux. Science **287:** 1604–1605

65 Takemura H., Hughes A. R., Thastrup O. and Putney J. W. Jr (1989) Activation of calcium entry by the tumor promoter, thapsigargin, in parotid acinar cells. Evidence that an intracellular calcium pool, and not an inositol phosphate, regulates calcium fluxes at the plasma membrane. J. Biol. Chem. **264:** 12266–12271

66 Hoth M. and Penner R. (1992) Depletion of intracellular calcium stores activates a calcium current in mast cells. Nature **355:** 353–355

67 Hoth M. and Penner R. (1993) Calcium release-activated calcium current in rat mast cells. J. Physiol. Lond. **465:** 359–386

68 Huber A., Sander P., Gobert A., Bähner M., Hermann R. and Paulsen R. (1996) The transient receptor potential protein (Trp), a putative store-operated Ca^{2+} channel essential for phosphoinositide-mediated photoreception, forms a signaling complex with NorpA, InaC and InaD. EMBO J. **15:** 7036–7045

69 Putney J. W. Jr (1999) Kissin' cousins: intimate plasma membrane-ER interactions underlie capacitative calcium entry. Cell **99:** 5–8

70 Jayaraman T., Ondriasova E., Ondrias K., Harnick D. J. and Marks A. R. (1995) The inositol 1,4,5-trisphosphate receptor is essential for T cell receptor signaling. Proc. Nat. Acad. Sci. USA **92:** 6007–6011

71 Sugawara H., Kurosaki M., Takata M. and Kurosaki T. (1997) Genetic evidence for involvement of type 1, type 2 and type 3 inositol 1,4,5-trisphosphate receptors in signal transduction through the B-cell antigen receptor. EMBO J. **16:** 3078–3088

72 Parekh A. B., Terlau H. and Stühmer W. (1993) Depletion of $InsP_3$ stores activates a Ca^{2+} and K^+ current by means of a phosphatase and a diffusible messenger. Nature **364:** 814–818

73 Ribeiro C. M. P., Reece J. and Putney J. W. Jr (1997) Role of the cytoskeleton in calcium signaling in NIH 3T3 cells. An intact cytoskeleton is required for agonist-induced $[Ca^{2+}]_i$ signaling, but not for capacitative calcium entry. J. Biol. Chem. **272:** 26555–26561

74 Holda J. R. and Blatter L. A. (1997) Capacitative calcium entry is inhibited in vascular endothelial cells by disruption of cytoskeletal microfilaments. FEBS Lett. **403:** 191–196

75 Alvarez J., Montero M. and Garcia-Sancho J. (1991) Cytochrome P-450 may link intracellular Ca^{2+} stores with plasma membrane Ca^{2+} influx. Biochem. J. **274:** 193–197

76 Graier W. F., Simecek S. and Sturek M. (1995) Cytochrome P450 mono-oxygenase-regulated signalling of Ca2 + entry in human and bovine endothelial cells. J. Physiol. (Lond.) **482:** 259–274

77 Rzigalinski B. A., Willoughby K. A., Hoffman S. W., Falck J. R. and Ellis E. F. (1999) Calcium influx factor, further evidence it is 5,6-epoxyeicosatrienoic acid. J. Biol. Chem. **274:** 175–185

78 Pandol S. J. and Schoeffield-Payne M. S. (1990) Cyclic GMP mediates the agonist-stimulated increase in plasma membrane calcium entry in the pancreatic acinar cell. J. Biol. Chem. **265:** 12846–12853

79 Xu X., Star R. A., Tortorici G. and Muallem S. (1994) Depletion of intracellular Ca^{2+} stores activates nitric-oxide synthase to generate cGMP and regulate Ca^{2+} influx. J. Biol. Chem. **269:** 12645–12653

80 Mathes C. and Thompson S. H. (1996) The nitric oxide/cGMP pathway couples muscarinic receptors to the activation of Ca^{2+} influx. J. Neurosci. **16:** 1702–1709

81 Randriamampita C. and Tsien R. Y. (1993) Emptying of intracellular Ca^{2+} stores releases a novel small messenger that stimulates Ca^{2+} influx. Nature **364:** 809–814

82 Csutora P., Su Z., Kim H. Y., Bugrim A., Cunningham K. W., Nuccitelli R. et al. (1999) Calcium influx factor is synthesized by yeast and mammalian cells depleted of organellar calcium stores. Proc. Natl. Acad. Sci. USA **96:** 121–126

83 Christian E. P., Spence K. T., Togo J. A., Dargis P. G. and Warawa E. (1996) Extracellular site for econazole-mediated block of Ca^{2+} release-activated Ca^{2+} current (I_{CRAC}) in T lymphocytes. Br. J. Pharmacol. **119:** 647–654

84 Vostal J. G. and Fratantoni J. C. (1993) Econazole inhibits thapsigargin-induced platelet calcium influx by mechanisms other than cytochrome P-450 inhibition. Biochem. J. **295:** 525–529

85 Bian X., Bird G. St. J. and Putney J. W. Jr (1996) Cyclic GMP is not required for capacitative calcium entry in JURKAT T-lymphocytes. Cell Calcium **19:** 351–354
86 Bird G. St. J., Bian X. and Putney J. W. Jr (1995) Calcium entry signal? Nature **373:** 481–482
87 Gilon P., Bird G. St. J., Bian X., Yakel J. L. and Putney J. W. Jr (1995) The Ca^{2+}-mobilizing actions of a Jurkat cell extract on mammalian cells and *Xenopus laevis* oocytes. J. Biol. Chem. **270:** 8050–8055
88 Lewis R. S. and Cahalan M. D. (1995) Potassium and calcium channels in lymphocytes. Ann. Rev. Immunol. **13:** 623–653
89 Lenormand P., Muldoon L. L., Enslen H., Rodland K. D. and Magun B. E. (1990) Two tumor promoters, 12-O-tetradecanoylphorbol-13-acetate and thapsigargin, act synergistically via distinct signaling pathways to stimulate gene expression. Cell Growth Differ. **1:** 627–635
90 Ammala C., Berggren P.-O., Bokvist K. and Rorsman P. (1992) Inhibition of L-type calcium channels by internal GTP[τS] in mouse pancreatic β cells. Pflüg. Arch. **420:** 72–77
91 Schonthal A., Sugarman J., Brown J. H., Hanley M. R. and Feramisco J. R. (1991) Regulation of c-*fos* and c-*jun* protooncogene expression by the Ca^{2+}-ATPase inhibitor thapsigargin. Proc. Natl. Acad. Sci. USA **88:** 7096–7100
92 Little E., Ramakrishnan M., Roy B., Gazit A. and Lee A. S. (1994) The glucose-regulated proteins (GRP78 and GRP94): functions, gene regulation and applications. Crit. Rev. Eukaryot. Gene Expr. **4:** 1–18
93 Milner R. E., Famulski K. S. and Michalak M. (1992) Calcium binding proteins in the sarcoplasmic/endoplasmic reticulum of muscle and nonmuscle cells. Mol. Cell. Biochem. **112:** 1–13
94 Drummond I. A. S., Lee A. S., Resendez E. Jr and Steinhardt R. A. (1987) Depletion of intracellular calcium stores by calcium ionophore A23187 induces the genes for glucose-regulated proteins in hamster fibroblasts. J. Biol. Chem. **262:** 12801–12805
95 Roy B. and Lee A. S. (1995) Transduction of calcium stress through interaction of the human transcription factor CBF with the proximal CCAAT regulatory element of the *grp78/BiP* promoter. Mol. Cell. Biol. **15:** 2263–2274
96 Li W. W., Hsiung Y., Zhou Y., Roy B. and Lee A. S. (1997) Induction of the mammalian GRP78/BiP gene by Ca^{2+} depletion and formation of aberrant proteins: activation of the conserved stress-inducible *grp* core promoter element by the human nuclear factor YY1. Mol.Cell. Biol. **17:** 54–60
97 Waser M., Mesaeli N., Spencer C. and Michalak M. (1997) Regulation of calreticulin gene expression by calcium. J. Cell Biol. **138:** 547–557
98 Kuo T. H., Liu B. F., Yu Y., Wuytack F., Raeymaekers L. and Tsang W. (1997) Co-ordinated regulation of the plasma membrane calcium pump and the sarco(endo)plasmic reticular pump gene expression by Ca^{2+}. Cell Calcium **21:** 399–408
99 Waldron R. T., Short A. D., Meadows J. J., Ghosh T. K. and Gill D. L. (1994) Endoplasmic reticulum calcium pump expression and control of cell growth. J. Biol. Chem. **268:** 11927–11933
100 Cheng G., Liu B.-F., Yu Y., Diglio C. and Kuo T. H. (1996) The exit from Go into the cell cycle requires and is controlled by sarco(endo)plasmic reticulum Ca^{2+} pump. Arch. Biochem. Biophys. **329:** 65–72
101 Tsukamoto A. and Kaneko Y. (1993) Thapsigargin, a Ca^{2+}-ATPase inhbitor, depletes the intracellular Ca^{2+} pool and induces apoptosis in human hepatoma cells. Cell Biol. Int. **17:** 969–970
102 Jiang S., Chow S. C., Nicotera P. and Orrenius S. (1994) Intracellular Ca^{2+} signals activate apoptosis in thymocytes: studies using the Ca^{2+}-ATPase inhibitor thapsigargin. Exp. Cell Res. **212:** 84–92
103 Bian X., Hughes F. M. Jr, Huang Y., Cidlowski J. A. and Putney J. W. Jr (1997) Roles of cytoplasmic Ca^{2+} and intracellular Ca^{2+} stores in induction and suppression of apoptosis in S49 cells. Am. J. Physiol. **272:** C1241–C1249
104 Qi X.-M., Simonson M. and Distelhorst C. W. (1997) Bcl-2 inhibits c-*fos* induction by calcium. Oncogene **15:** 2849–2853
105 Ghosh A., Ginty D. D., Bading H. and Greenberg M. E. (1994) Calcium regulation of gene expression in neuronal cells. J. Neurobiol. **25:** 294–303
106 Bading H., Hardingham G. E., Johnson C. M. and Chawla S. (1997) Gene regulation by nuclear and cytoplasmic calcium signals. Biochem. Biophys. Res. Comm. **236:** 541–543
107 Husain M., Bein K., Jiang L., Alper S. L., Simons M. and Rosenberg R. D. (1997) c-Myb-dependent cell cycle progression and Ca^{2+} storage in cultured vascular smooth muscle cells. Circ. Res. **80:** 617–626
108 Gissel C., Doutheil J. and Paschen W. (1997) Activation of heme oxygenase-1 expression by disturbance of endoplasmic reticulum calcium homeostasis in rat neuronal cell culture. Neurosci. Lett. **231:** 75–78
109 Sudo Y. and Mariash C. N. (1996) Lowering glucose depletes a thapsigargin-sensitive calcium pool and inhibits transcription of the S14 gene. Endocrinology **137:** 4677–4684
110 Caron-Leslie L. M. and Cidlowski J. A. (1991) Similar actions of glucocortcoids and calcium on the regulation of apoptosis in S49 cells. Mol. Endocrinol. **5:** 1169–1179
111 Nicotera P., Bellomo G. and Orrenius S. (1992) Calcium-mediated mechanisms in chemically induced cell-death. Ann. Rev. Pharmacol. Toxicol. **32:** 449–470
112 Furuya Y., Lundmo P., Short A. D., Gill D. L. and Isaacs J. T. (1994) The role of calcium, pH, and cell proliferation in the programmed (apoptotic) death of androgen-independent prostatic cancer cells induced by thapsigargin. Cancer Res. **54:** 6167–6175
113 Zhu W.-H. and Loh T.-T. (1995) Roles of calcium in the regulation of apoptosis in HL-60 promyelocytic leukemia cells. Life Sci. **57:** 2091–2099
114 Kaiser N. and Edelman I. S. (1977) Calcium dependence of glucocorticoid-induced lymphocytolysis. Proc. Natl. Acad. Sci. USA **74:** 638–642
115 McConkey D. J., Nicotera P., Hartzell P., Bellomo G., Wyllie A. H. and Orrenius S. (1989) Glucocorticoids activate a suicide process in thymocytes through an elevation of cytosolic Ca^{2+} concentration. Arch. Biochem. Biophys. **269:** 365–370
116 McConkey D. J., Aguilar-Santelises M., Hartzell P., Mellstedt H., Orrenius S. and Jondal M. (1991) Induction of DNA fragmentation in chronic B-lymphocytic leukemia cells. J. Immunol. **146:** 1072–1076
117 Story M. D., Stephens L. C., Tomasovic S. P. and Meyn R. E. (1992) A role for calcium in regulating apoptosis in rat thymocytes irradiated in vitro. Int. J. Radiat. Biol. **61:** 243–251
118 Robertson L. E., Chubb S., Meyn R. E., Story M., Ford R., Hittelman W. N. et al. (1993) Induction of apoptotic cell death in chronic lymphocytic leukemia by 2-chloro-2′-deoxyadenosine and 9-beta-arabinosyl-2′-fluoradenine. Blood **81:** 143–150
119 Dowd D. R., MacDonald P. N., Komm B. S., Haussler M. R. and Miesfeld R. (1992) Stable expression of the calbindin-D28K complementary DNA interferes with the apoptotic pathway in lymphocytes. Mol. Endocrinol. **6:** 1843–1848
120 Martikainen P. and Isaacs J. (1990) Role of calcium in the programmed death of rat prostatic glandular cells. Prostate **17:** 175–187
121 Klee, C. B. and Newton, D. L. (1985) Calmodulin: an overview. In: Control and Manipulation of Calcium Movement, pp. 131–145, Parratt J. R. (ed.), Raven Press, New York
122 Bachs O., Agell N. and Carafoli E. (1992) Calcium and calmodulin function in the cell nucleus. Biochim. Biophys. Acta **1113:** 259–270
123 Lu K. P. and Means A. R. (1993) Regulation of the cell cycle by calcium and calmodulin. Endocrine Rev. **14:** 40–58

124 Dowd D. R., MacDonald P. N., Komm B. S., Haussler M. R. and Miesfeld R. (1991) Evidence for early induction of calmodulin gene expression in lymphocytes undergoing glucocorticoid-mediated apoptosis. J. Biol. Chem. **266:** 18423–18426

125 Baffy G., Miyashita T., Williamson J. R. and Reed J. C. (1993) Apoptosis induced by withdrawal of interleukin-3 (IL-3) from an IL-3-dependent hematopoietic cell line is associated with repartitioning of intracellular calcium and is blocked by enforced Bcl-2 oncoprotein production. J. Biol. Chem. **268:** 6511–6519

126 Lam M., Dubyak G., Chen L., Nuñez G., Miesfeld R. L. and Distelhorst C. W. (1994) Evidence that BCL-2 represses apoptosis by regulating endoplasmic reticulum-associated Ca^{2+} fluxes. Proc. Natl. Acad. Sci. USA **91:** 6569–6573

127 Lam M., Dubyak G. and Distelhorst C. W. (1993) Effect of glucocorticosteroid treatment on intracellular calcium homeostasis in mouse lymphoma cells. Mol. Endocrinol. **7:** 686–693

128 Zhivotovsky B., Cedervall B., Jiang S., Nicotera P. and Orrenius S. (1994) Involvement of Ca^{2+} in the formation of high molecular weight DNA fragments in thymocyte apoptosis. Biochem. Biophys. Res. Comm. **202:** 120–127

129 Sun X.-M. and Cohen G. M. (1994) Mg^{2+}-dependent cleavage of DNA into kilobase pair fragments is responsible for the initial degradation of DNA in apoptosis. J. Biol. Chem. **269:** 14857–14860

130 Nicotera P. and Orrenius S. (1998) The role of calcium in apoptosis. Cell Calcium **23:** 173–180

131 Wong W. L., Brostrom M. A., Kuznetsov G., Gmitter-Yellen D. and Brostrom C. O. (1993) Inhibition of protein synthesis and early protein processing by thapsigargin in cultured cells. Biochem. J. **289:** 71–79

132 Kelley L. L., Koury M. J. and Bondurant M. C. (1992) Regulation of programmed death in erythroid progenitor cells by erythropoietin: effects of calcium and of protein and RNA syntheses. J. Cell. Physiol. **151:** 487–496

133 Caron-Leslie L. M., Evans R. B. and Cidlowski J. A. (1994) Bcl-2 inhibits glucocorticoid-induced apoptosis but only partially blocks ionophore or cycloheximide-regulated apoptosis in S49 cells. FASEB J. **8:** 639–645

134 Pinton P., Ferrari D., Magalhaes P., Schulze-Osthoff K., Di Virgilio F., Pozzan T. et al. (2000) Reduced loading of intracellular Ca^{2+} stores and downregulation of capacitative Ca^{2+} influx in Bcl-2-overexpressing cells. J. Cell Biol. **148:** 857–862

135 Khan A. A., Soloski J. J., Sharp A. H., Schilling G., Sabatini D. M., Li S. et al. (1996) Lymphocyte apoptosis: mediation by increased type 3 inositol 1,4,5-trisphosphate receptor. Science **273:** 503–507

136 Putney J. W. Jr (1997) The type 3 inositol 1,4,5-trisphosphate receptor and capacitative calcium entry. Cell Calcium **21:** 257–261

137 Jayaraman T. and Marks A. R. (1997) T cells deficient in inositol 1,4,5-trisphosphate receptor are resistant to apoptosis. Mol. Cell. Biol. **17:** 3005–3012

138 Hockenberry D. M., Nunez G., Milliman C., Schreiber R. D. and Korsmeyer S. J. (1990) Bcl2 is an inner mitochondrial membrane protein that blocks programmed cell death. Nature **348:** 334–336

139 Alnemri E. S., Fernandes T. F., Haldar S., Croce C. M. and Litwack G. (1992) Involvement of BCL-2 in glucocorticoid-induced apoptosis of human pre-B-leukemia. Cancer Res. **52:** 491–495

140 Korsmeyer S. J. (1995) Regulators of cell death. Trends Genet. **11:** 101–105

141 Shimizu S., Narita M. and Tsujimoto Y. (1999) Bcl-2 family proteins regulate the release of apoptogenic cytochrome *c* by the mitochondrial channel VDAC. Nature **399:** 483–487

142 Lam M., Bhat M. B., Nuñez G., Ma J. and Distelhorst C. W. (1998) Regulation of Bcl-xl channel activity by calcium. J. Biol. Chem. **273:** 17307–17310

143 Antonsson B., Conti F., Ciavatta A., Montessuit S., Lewis S., Martinou I. et al. (1997) Inhibition of BAX channel-forming activity by BCL-2. Science **277:** 370–372

144 He H., Lam M., McCormick T. S. and Distelhorst C. W. (1997) Maintenance of calcium homeostasis in the endoplasmic reticulum by Bcl-2. J. Cell Biol. **138:** 1219–1228

145 Kuo T. H., Kim H.-R. C., Zhu L., Yu Y., Lin H.-M. and Tsang W. (1998) Modulation of endoplasmic reticulum calcium pump by Bcl-2. Oncogene **17:** 1903–1910

146 Terasaki M. and Jaffe L. A. (1991) Organization of the sea urchin egg endoplasmic reticulum and its reorganization at fertilization. J. Cell Biol. **114:** 929–940

147 Terasaki M. (1989) Fluorescent labeling of endoplasmic reticulum. Methods Cell Biol. **29:** 125–135

148 Jaffe L. A. and Terasaki M. (1993) Structural changes of the endoplasmic reticulum of sea urchin eggs during fertilization. Dev. Biol. **156:** 566–573

149 Jaffe L. A. and Terasaki M. (1994) Structural changes in the endoplasmic reticulum of starfish oocytes during meiotic maturation and fertilization. Dev. Biol. **164:** 579–587

150 Terasaki M., Jaffe L. A., Hunnicut G. R. and Hammer J. A. III (1996) Structural change of the endoplasmic reticulum during fertilization: evidence for loss of membrane continuity using the green fluorescent protein. Dev. Biol. **179:** 320–328

151 Subramanian K. and Meyer T. (1997) Calcium-induced restructuring of nuclear envelope and endoplasmic reticulum calcium stores. Cell **89:** 963–971

152 Ribeiro C. M. P., McKay R. R., Hosoki E. and Putney J. W. Jr. (2000) Effects of elevated cytoplasmic calcium and protein kinase C on endoplasmic reticulum structure and function in HEK293 cells. Cell Calcium **27:** 175–185

153 Drummond A. H. (1985) Bidirectional control of cytosolic free calcium by thyrotropin-releasing hormone in pituitary cells. Nature **315:** 752–755

154 Hepler J. R., Earp H. S. and Harden T. K. (1988) Long-term phorbol ester treatment down-regulates protein kinase C and sensitizes the phosphoinositide signalling pathway to hormone and growth factor stimulation. Evidence for a role of protein kinase C in agonist-induced desensitization. J. Biol. Chem. **263:** 7610–7619

155 Bird G. S., Rossier M. F., Obie J. F. and Putney J. W. Jr. (1993) Sinusoidal oscillations in intracellular calcium requiring negative feedback by protein kinase C. J. Biol. Chem. **268:** 8425–8428

156 Petersen C. C. H. and Berridge M. J. (1994) The regulation of capacitative calcium entry by calcium and protein kinase C in *Xenopus* oocytes. J. Biol. Chem. **269:** 32246–32253

157 Bode H.-P. and Göke B. (1994) Protein kinase C activates capacitative calcium entry in the insulin secreting cell line RINm5F. FEBS Letters **339:** 307–311

158 Montero M., Garcia-Sancho J. and Alvarez J. (1993) Inhibition of the calcium store-operated calcium entry pathway by chemotactic peptide and by phorbol ester develops gradually and independently along differentiation of HL60 cells. J. Biol. Chem. **268:** 26911–26919

159 Törnquist K. (1993) Modulatory effect of protein kinase C on thapsigargin-induced calcium entry in thyroid FRTL-5 cells. Biochem. J. **290:** 443–447

160 Montero M., Garcia-Sancho J. and Alvarez J. (1994) Phosphorylation downregulates the store-operated Ca^{2+} entry pathway of human neutrophils. J. Biol. Chem. **269:** 3963–3967

161 Parekh A. B. and Penner R. (1995) Depletion-activated calcium current is inhibited by protein kinase in RBL-2H3 cells. Proc. Nat. Acad. Sci. USA **92:** 7907–7911

162 Ribeiro C. M. P. and Putney J. W. Jr (1996) Differential effects of protein kinase C activation on calcium storage and capacitative calcium entry in NIH 3T3 cells. J. Biol. Chem. **271:** 21522–21528

163 Baranska J., Chaban V., Czarny M. and Sabala P. (1995) Changes in Ca^{2+} concentration in phorbol ester and thapsigargin treated glioma C6 cells. The role of protein kinase C in regulation of Ca^{2+} entry. Cell Calcium **17:** 207–215

164 Murphy C. T., Bullock A. J. and Westwick J. (1996) A role for protein phosphorylation in modulating Ca^{2+} elevation in rabbit platelets treated with thapsigargin. Biochem. J. **313:** 83–89

165 Goodnight J., Mischak H., Kolch W. and Mushinski J. F. (1995) Immunocytochemical localization of eight protein kinase C isozymes overexpressed in NIH 3T3 fibroblasts. J. Biol. Chem. **270:** 9991–10001

166 Putney J. W. Jr (1993) Excitement about calcium signaling in inexcitable cells. Science **262:** 676–678