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REGULATION OF INOSITOL 1,4,5-TRISPHOSPHATE RECEPTORS

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Summary

Inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] is a soluble second messenger responsible for the generation of highly organized Ca^{2+} signals in a variety of cell types. These Ca^{2+} signals control many cellular responses, including cell growth, fertilization, smooth muscle contraction and secretion. Ins(1,4,5)P_3 is produced at the plasma membrane following receptor activation, but rapidly diffuses into the cytosol, where it binds to specific receptors through which it mobilizes intracellular Ca^{2+} stores. The actions of Ins(1,4,5)P_3 within cells are tightly controlled: enzymes control the rapid generation and metabolism of Ins(1,4,5)P_3 following receptor activation; multiple Ins(1,4,5)P_3 receptor subtypes and splice variants exist, some of which are differentially expressed between cell types and at different stages of development; and Ins(1,4,5)P_3 receptors are the targets for a number of allosteric regulators, including protein kinases, ATP and divalent cations. Understanding how cells control the Ca^{2+}-mobilizing activity of Ins(1,4,5)P_3 will be important if we are to unravel the mechanisms that underlie the complex arrangements of Ca^{2+} signals.

Introduction

Cellular responses as diverse as fertilization, cell growth and development, secretion, muscle contraction, sensory perception and neuromodulation depend critically on elevations in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (Berridge, 1993). The introduction of sophisticated imaging techniques has revealed astonishing complexities in the temporal and spatial characteristics of these Ca^{2+} elevations in single cells (see review by Cheek and Barry, 1993). Repetitive, transient elevations in intracellular $[Ca^{2+}]$ (Ca^{2+} spikes or oscillations) have been recorded in a number of cell types (see review by Thorn, 1993); these can be arranged spatially as repetitive Ca^{2+} waves originating from a specific point within the cytosol (see review by Gillot and Whitaker, 1993), or even as regenerative Ca^{2+} spirals (Lechleiter *et al.* 1991).

Whilst the organization of these signals has been extensively characterized, a more difficult problem has been to establish their physiological significance. A recurring criticism is that the techniques employed to measure Ca^{2+} signals, such as the incorporation of Fura-2, a mobile Ca^{2+} buffer and fluorescent Ca^{2+}-indicator dye, into the cytosol may themselves contribute to the complex spatial organization. A few examples have been reported, however, in which oscillatory Ca^{2+} signals correlate with periodic cellular

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responses. In monolayers of ciliated airway epithelia, Ca^{2+} waves that propagate across, and between, cells correlate with waves of increased beating-frequency of cilia (Sanderson *et al.* 1990), a response which may be important for the removal of foreign objects from the airways. In rat gonadotrophs, Ca^{2+} oscillations have been shown to correlate with periodic elevations in membrane capacitance, reflecting exocytotic vesicle fusion (Tse *et al.* 1993).

Rises in intracellular Ca^{2+} concentration are generated by a combination of the release of stored Ca^{2+} from organelles and the stimulation of Ca^{2+} influx across the plasma membrane, although the relative contributions from these two sources of Ca^{2+} varies between tissues. In skeletal and cardiac muscle, for example, Ca^{2+} is stored in the sarcoplasmic reticulum (SR) from which it is released through ryanodine-sensitive Ca^{2+} channels (ryanodine receptors, RyRs) following depolarization of T-tubules: however, whilst skeletal muscle RyRs are activated directly by voltage-sensing dihydropyridine receptors in the T-tubular membrane, release of Ca^{2+} through cardiac muscle RyRs is triggered by the Ca^{2+} that enters the cells in response to depolarization (reviewed by Sorrentino and Volpe, 1993).

In many cell types, intracellular Ca^{2+} stores are mobilized by the soluble second messenger inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3]. Ins(1,4,5)P_3 is produced at the plasma membrane following receptor activation and mobilizes Ca^{2+} through specific receptors that are structurally similar to the RyRs of skeletal and cardiac muscle (Berridge and Irvine, 1984, 1989); the depletion of the Ca^{2+} stores by Ins(1,4,5)P_3 is thought to provide a signal for Ca^{2+} influx across the plasma membrane (Putney, 1986). Initially, Ins(1,4,5)P_3 was proposed to provide only the initial trigger for the onset of Ca^{2+} responses (Oron *et al.* 1985; Parker and Miledi, 1986; Berridge and Galione, 1988), but growing evidence now supports a role for Ins(1,4,5)P_3 in the temporal and spatial organization of Ca^{2+} signals (Harootunian *et al.* 1991; DeLisle and Welsh, 1992; Miyazaki *et al.* 1992) and possibly even a direct role in the stimulation of Ca^{2+} influx across the plasma membrane (Irvine, 1991). Indeed, with the identification of multiple Ins(1,4,5)P_3 receptor subtypes, and the growing number of cytosolic factors known to regulate these receptors allosterically, it is likely that the control of Ins(1,4,5)P_3-stimulated Ca^{2+} mobilization is important in contributing to the organization and diversity of Ca^{2+} signals.

Generation and metabolism of Ins(1,4,5)P_3

Ins(1,4,5)P_3 levels within cells peak during the first few seconds of agonist stimulation, after which they are rapidly reduced either to basal levels or, in the continued presence of agonist, to a slightly elevated plateau. These rapid, transient changes in Ins(1,4,5)P_3 concentration result from the competing metabolic pathways associated with Ins(1,4,5)P_3 generation and Ins(1,4,5)P_3 metabolism.

*Ins(1,4,5)*P$_3$ *generation*

Ins(1,4,5)P_3 is generated in cells by the receptor-mediated activation of certain phospholipase C (PLC) isoforms (Fig. 1). Two classes of cell-surface receptor are involved. Receptors coupled to GTP-binding (G) proteins of the G_q family undergo a conformational change following agonist binding which allows them to interact with, and

activate, the G-protein. An active subunit of the G-protein stimulates PLC-β. Receptors with intrinsic tyrosine kinase activity dimerize in response to agonist stimulation: the dimers phosphorylate each other on tyrosine residues within the cytosolic domains of the receptors, and these phosphotyrosine residues provide a site to which PLC-γ binds and becomes activated. Both PLC-β and PLC-γ hydrolyse the membrane-associated lipid, phosphatidylinositol 4,5-bisphosphate, to produce 1,2-diacylglycerol and Ins(1,4,5)P_3. 1,2-Diacylglycerol remains in the plasma membrane where it activates proteins kinase C (Nishizuka, 1988), whereas Ins(1,4,5)P_3 diffuses into the cytosol and binds to specific receptors through which it mobilizes intracellular Ca^{2+} stores (Taylor and Richardson, 1991).

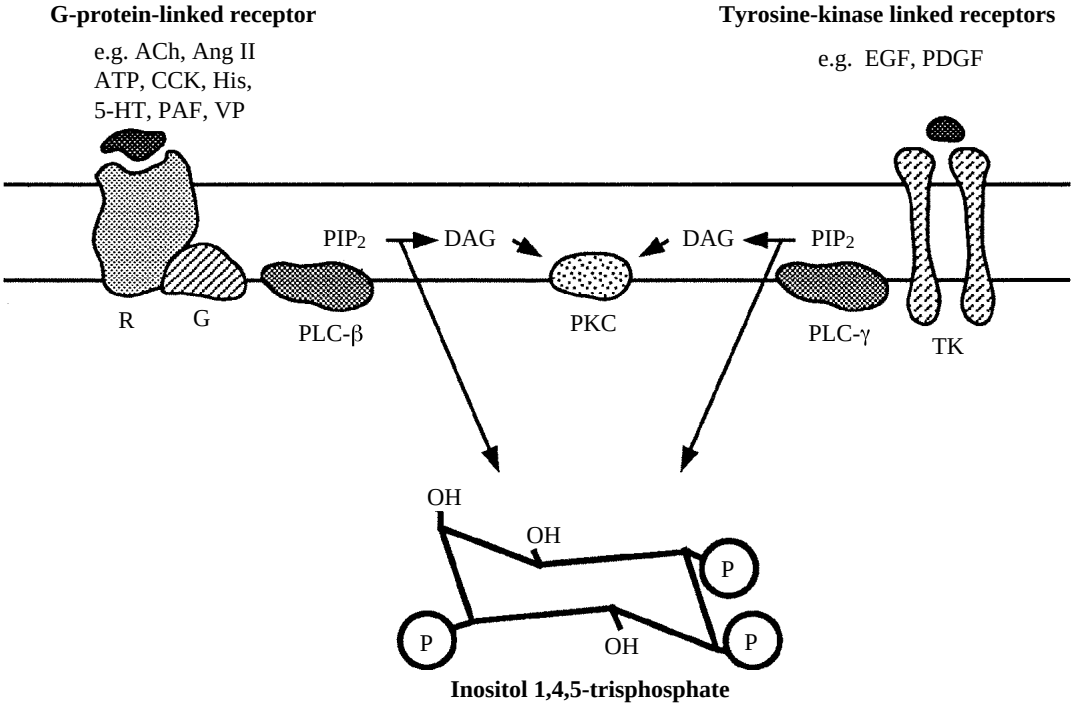

Fig. 1. Receptor-stimulated Ins(1,4,5)P_3 formation. Ins(1,4,5)P_3 is generated by the receptor-mediated activation of phospholipase C (PLC), which hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP_2) into 1,2-diacylglycerol (DAG) and Ins(1,4,5)P_3. DAG activates proteins kinase C (PKC) in the plasma membrane, whereas Ins(1,4,5)P_3 binds to specific receptors on Ca^{2+}-storing organelles and mobilizes intracellular Ca^{2+} stores. Various isoforms of PLC are activated by different classes of cell surface receptor. Receptors (R) coupled to GTP-binding proteins (G) of the G_q family stimulate PLC-β *via* activation of the G-protein. Receptors with intrinsic tyrosine kinase activity (TK) dimerize in response to agonist-stimulation: the dimers phosphorylate each other on cytosolic tyrosine residues, and these phosphotyrosine residues provide sites to which PLC-γ binds and becomes activated. ACh, acetylcholine; Ang II, angiotensin II; CCK, cholecystokinin; His, histamine; 5-HT, 5-hydroxytryptamine; PAF, platelet-activating factor; VP, vasopressin; EGF, epidermal growth factor; PDGF, platelet-derived growth factor.

Ins(1,4,5)P_3 *metabolism*

Ins(1,4,5)P_3 is inactivated by two metabolic pathways. It is either phosphorylated by a 3-kinase to yield inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P_4] (Batty *et al.* 1985; Irvine *et al.* 1986) or dephosphorylated by a 5-phosphatase to give inositol 1,4-bisphosphate [Ins(1,4)P_2] (Downes *et al.* 1982; Berridge *et al.* 1983). Ins(1,3,4,5)P_4 may itself regulate intracellular Ca^{2+} concentration by promoting Ca^{2+} sequestration (Hill *et al.* 1988), by synergizing with Ins(1,4,5)P_3 to mobilize intracellular Ca^{2+} stores (Cullen *et al.* 1990; Gawler *et al.* 1990) or by regulating agonist-stimulated Ca^{2+} entry across the plasma membrane (Irvine and Moor, 1986). The presence of specific Ins(1,3,4,5)P_4-binding sites in a number of tissues further supports a role for Ins(1,3,4,5)P_4 in the phosphoinositide signalling pathway (Bradford and Irvine, 1987; Theibert *et al.* 1987; Enyedi and Williams, 1988; Donié and Reiser, 1989). Ins(1,4)P_2, in contrast, probably plays no part in signal transduction and is rapidly metabolized to inositol (Storey *et al.* 1984).

Ins(1,4,5)P_3 receptors

Identification

Evidence from a variety of sources has indicated that Ins(1,4,5)P_3 binds to a specific Ins(1,4,5)P_3 receptor/Ca^{2+} channel complex located on intracellular Ca^{2+} stores where it directly stimulates release of Ca^{2+} into the cytosol. Comparison of the abilities of various inositol phosphates to release Ca^{2+} from permeabilized cells demonstrated a structure–activity relationship indicative of the binding of inositol phosphates to a specific receptor (Burgess *et al.* 1984). Ins(2,4,5)P_3 and Ins(4,5)P_2 were both able to mobilize stored Ca^{2+}, although less potently than Ins(1,4,5)P_3, whereas Ins(1,4)P_2 was without effect. Thus, the ability of the inositol phosphates to mobilize intracellular Ca^{2+} depends on the presence of phosphate groups at both the 4 and 5 positions of the inositol ring and is enhanced by a phosphate at the 1 position.

High-affinity, saturable binding sites specific for Ins(1,4,5)P_3 have been identified in a number of tissues (Baukal *et al.* 1985; Spät *et al.* 1986; Worley *et al.* 1987; Guillemette *et al.* 1987), and binding of Ins(1,4,5)P_3 to these sites has been shown to correlate with Ins(1,4,5)P_3-stimulated Ca^{2+} mobilization (Spät *et al.* 1986; Guillemette *et al.* 1987). Ins(1,4,5)P_3 receptors have since been purified from a variety of tissues and species (Supattapone *et al.* 1988*b*; Maeda *et al.* 1991; Mourey *et al.* 1990; Chadwick *et al.* 1990; Parys *et al.* 1992). When reconstituted into lipid vesicles, the purified receptor mediates Ca^{2+} flux in response to Ins(1,4,5)P_3, providing firm evidence that both the Ins(1,4,5)P_3-binding site and the Ca^{2+} channel reside in the same protein complex (Ferris *et al.* 1989).

Structure

The structure and membrane topology of Ins(1,4,5)P_3 receptors is shown in Fig. 2. Each receptor consists of four similar subunits which are non-covalently associated (Mignery *et al.* 1990; Maeda *et al.* 1991). From electron microscopy of purified receptors, the four subunits have been shown to adopt a four-leaf-clover-like structure (Chadwick *et al.* 1990), at the centre of which is thought to be the Ca^{2+} channel itself. This bears a

marked resemblance to the structure of ryanodine receptors, the Ca^{2+} release channels of skeletal and cardiac muscle (Lai *et al.* 1988), and indeed these proteins show considerable homology in their primary sequences (Mignery *et al.* 1989), higher-order structures and in allosteric regulation (see below). The two receptors may be derived from a common ancestral gene. Our idea of how these intracellular Ca^{2+} channels operate has been greatly influenced by the parallels that appear to exist between the two proteins.

Immunolocalization studies have revealed that both the amino and carboxy termini of the Ins(1,4,5)P_3 receptor subunits are cytosolic (Mignery *et al.* 1989; Nakade *et al.* 1991). Deletion of amino acid residues from the N-terminal region of the receptor either abolishes (Mignery *et al.* 1990) or greatly reduces (Miyawaki *et al.* 1991) binding of

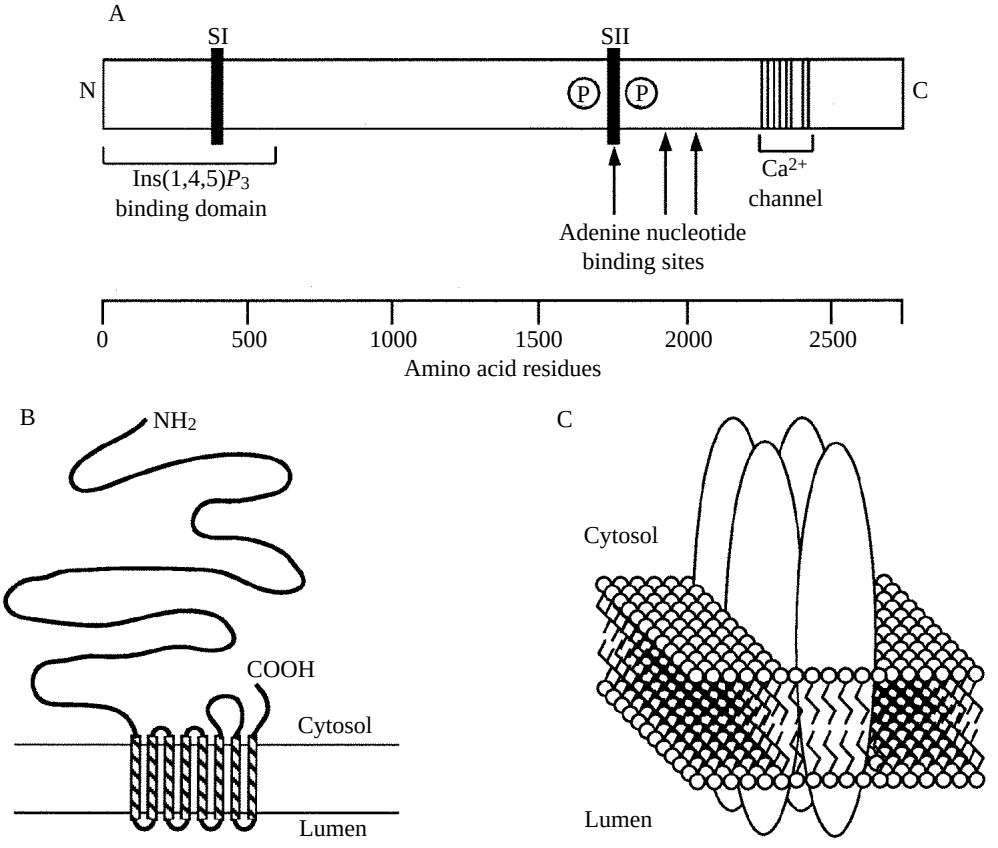

Fig. 2. Ins(1,4,5)P_3 receptor structure and membrane topology. (A) Primary sequence of the 2749 amino-acid rat type 1 Ins(1,4,5)P_3 receptor, showing the location of the Ins(1,4,5)P_3-binding domain, the Ca^{2+} channel, alternatively spliced sequences (SI and SII), three putative adenine nucleotide binding sites, and two sites (P) at which the receptor is phosphorylated by cyclic-AMP-dependent protein kinase. (B) Each subunit of the Ins(1,4,5)P_3 receptor is anchored into the membrane of intracellular Ca^{2+} stores by 6–8 transmembrane regions close to the C terminus. Both the short C-terminal tail and the long N-terminal tail are cytosolic. (C) Functional Ins(1,4,5)P_3 receptors are made up of four non-covalently associated subunits; at the centre of these is the Ca^{2+} channel.

Ins(1,4,5)P_3, suggesting that the Ins(1,4,5)P_3-binding site is located within the first 788 residues of the N terminus of each subunit. The Ca^{2+} channel is thought to reside close to the C terminus of the receptor and indeed monoclonal antibodies directed against the C terminus have been shown to inhibit Ca^{2+} release (Nakade *et al.* 1991). Hydrophobicity analysis has identified between six and eight membrane-spanning domains close to the C terminus (Mignery *et al.* 1990; Yoshikawa *et al.* 1992), which are important for anchoring the protein into membranes (Mignery and Südhof, 1990). The four putative transmembrane regions closest to the C terminus, which are flanked by a large number of net negative charges, show the highest degree of homology with ryanodine receptors, and they are therefore presumed to be important in the gating of Ca^{2+} (Mignery *et al.* 1990). The C terminus is also thought to be important in the formation of tetrameric complexes, since deletion of this portion of the receptor results in the formation of monomeric Ins(1,4,5)P_3-binding proteins (Mignery and Südhof, 1990; Miyawaki *et al.* 1991).

Located between the N-terminal Ins(1,4,5)P_3-binding domain and the C-terminal Ca^{2+} channel is a stretch of more than 1500 amino acid residues which is the target of a number of allosteric regulators of the Ins(1,4,5)P_3 receptor (discussed below). When Ins(1,4,5)P_3 binds to the N terminus, a large conformational change ensues (Mignery and Südhof, 1990) which is thought to link Ins(1,4,5)P_3 binding, *via* this long coupling domain, to opening of the Ca^{2+} channel. This may be an oversimplification, however, since we do not yet know how the Ins(1,4,5)P_3 receptor folds within the membrane of intracellular stores, and it is possible that regions further from the N terminus also influence Ins(1,4,5)P_3 binding (see Furuichi *et al.* 1989).

Localization

Ins(1,4,5)P_3 receptors are highly concentrated in the Purkinje cells of the cerebellum (the physiological significance of such high receptor density is not understood), with lower levels being found in other regions of the brain (Worley *et al.* 1987, 1989) and in a variety of peripheral tissues (Chadwick *et al.* 1990; Guillemette *et al.* 1990; Sharp *et al.* 1992). At a subcellular level, immunohistochemical studies in Purkinje cells, *Xenopus* oocytes and pancreatic epithelial cells have localized Ins(1,4,5)P_3 receptors to the rough and smooth endoplasmic reticula and the subplasmalemmal cisternae, with conflicting reports as to the presence of Ins(1,4,5)P_3 receptors in the nucleus and an apparent absence of receptors in the mitochondria and Golgi cisternae (Ross *et al.* 1989; Maeda *et al.* 1990; Parys *et al.* 1992; Sharp *et al.* 1992). More precise localization has been achieved using immunogold labelling, resulting in the localization of receptors to specific subcompartments of the endoplasmic reticulum (Satoh *et al.* 1990). Consistent with the Ins(1,4,5)P_3-sensitive Ca^{2+} stores residing in specialized regions of the endoplasmic reticulum, vasopressin, an agonist linked to phosphoinositide metabolism, reduces the Ca^{2+} content of the rough endoplasmic reticulum in rat liver (Bond *et al.* 1987).

In some cells, Ins(1,4,5)P_3 receptors have been found to be associated with the plasma membrane (Kuno and Gardner, 1987; Khan *et al.* 1992), prompting speculation that Ins(1,4,5)P_3 may play a direct role in regulating hormone-dependent Ca^{2+} influx. These plasma-membrane-associated Ins(1,4,5)P_3 receptors appear to show less specificity for Ins(1,4,5)P_3 over other inositol phosphates compared with Ins(1,4,5)P_3 receptors

localized in the endoplasmic reticulum (Kalinoski *et al.* 1992). Attention has therefore been drawn towards the Ins(1,4,5)P_3 metabolite, Ins(1,3,4,5)P_4, as a candidate for a physiological regulator of Ca^{2+} influx (Irvine, 1991). Interestingly, Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 appear to act synergistically to stimulate Ca^{2+} influx into sea urchin eggs (Irvine and Moor, 1986) and lacrimal cells (Morris *et al.* 1987), and in endothelial cells a Ca^{2+} channel within the plasma membrane appears to be activated by Ins(1,3,4,5)P_4, but is insensitive to Ins(1,4,5)P_3 (Lückhoff and Clapham, 1992).

The mechanism(s) underlying receptor-mediated Ca^{2+} influx remain elusive, but it is clear that the products of phosphoinositide metabolism are likely to feature prominently in at least some cell types.

Ins(1,4,5)P_3 receptor diversity

*Ins(1,4,5)*P$_3$ *receptor subtypes*

Two distinct genes have been identified in rodent cerebellum that encode full-length sequences for Ins(1,4,5)P_3 receptors (Furuichi *et al.* 1989; Mignery *et al.* 1990; Südhof *et al.* 1991). The type 1 and type 2 receptors (2749 and 2701 amino acids in length respectively) are 69% identical in their amino acid sequences and are structurally very similar, both to each other and to the ryanodine receptor Ca^{2+} channel of skeletal muscle (Takeshima *et al.* 1989). The type 2 receptor is much less abundant than the type 1 receptor (Südhof *et al.* 1991). Significantly, the ligand-binding site and most of the transmembrane domains comprising the Ca^{2+} channel appear to be the most conserved regions between the receptor subtypes, whereas the coupling domain shows the greatest diversity (Südhof *et al.* 1991). The major functional differences between the two receptors could therefore be associated with their respective patterns of allosteric regulation. Partial sequences have now been obtained indicating at least one further subtype (Ross *et al.* 1992), which suggests that a large family of Ins(1,4,5)P_3 receptors may exist.

Full-length sequences encoding Ins(1,4,5)P_3 receptors from *Xenopus* oocytes (Kume *et al.* 1993) and *Drosophila melanogaster* (Yoshikawa *et al.* 1992) have now been determined. The *Xenopus* receptor appears to be a species variant of the type 1 receptor, displaying 90% sequence homology with the mouse cerebellar type 1 receptor, whereas the *Drosophila* receptor differs from both the type 1 and type 2 receptors to a similar extent (Yoshikawa *et al.* 1992). Once again, the receptor from *Drosophila* shows considerable sequence homology with the mouse type 1 and *Xenopus* receptors in both the ligand-binding domain and Ca^{2+} channel region, but displays considerable diversity in the coupling domain. Most significantly, the *Drosophila* receptor lacks consensus sequences for phosphorylation in the coupling domain, indicating that it is not a substrate for regulation by cyclic-AMP-dependent protein kinase (PKA).

Splice variants

Ins(1,4,5)P_3 receptor diversity can also be generated by differential splicing of receptor transcripts. Two major splice sequences, SI and SII, have been identified within the type 1 receptor. SI encodes a 15 amino-acid sequence located within the N-terminal Ins(1,4,5)P_3-

binding domain (Mignery *et al.* 1990; Nakagawa *et al.* 1991) and may therefore influence the binding characteristics of Ins(1,4,5)P_3. SII is larger, encoding 40 amino acids within the regulatory domain of the receptor (Danoff *et al.* 1991), and is made up of three smaller splice variants (A, B and C: 23, 1 and 16 residues respectively) which can each be independently removed to generate a series of different Ins(1,4,5)P_3 receptor transcripts (Nakagawa *et al.* 1991). The SII splice sequence is located between two PKA phosphorylation consensus sequences (Danoff *et al.* 1991), and it is therefore not surprising that receptors that differ in the expression of this splice sequence also appear to differ in their patterns of phosphorylation by PKA (see below; Danoff *et al.* 1991).

At present we have little idea as to the physiological significance of these multiple receptor transcripts, although the conservation of alternative splice events between species (Danoff *et al.* 1991) suggests an important role in the regulation of Ins(1,4,5)P_3 receptor activity. Recently, different Ins(1,4,5)P_3 receptor splice variants have been shown to be expressed in both a developmentally specific and tissue-specific manner (Danoff *et al.* 1991; Nakagawa *et al.* 1991). The formation of synapses between Purkinje cells and granule cells during cerebellar development, for example, coincides with peak expression of receptors containing the SI splice sequence, whereas other forms of the receptor show a progressively increasing level of expression throughout this period of development (Nakagawa *et al.* 1991). In mouse, Ins(1,4,5)P_3 receptors containing the SII splice sequence are exclusively localized in neuronal tissue, whereas the shorter forms of the receptor lacking this SII domain are found largely in the periphery (Danoff *et al.* 1991). The predominance of SII-containing receptors in neuronal tissue is, however, not common to all species, since Ins(1,4,5)P_3 receptors cloned from *Drosophila* head lack the SII splice sequence (Kume *et al.* 1993).

It is clear that a number of subtypes and isoforms of the Ins(1,4,5)P_3 receptor exist and that these can be differentially expressed both in different tissues and at different stages of development. Furthermore, cerebellar Purkinje cells are now known to express at least two, and possibly three, distinct Ins(1,4,5)P_3 receptors (Ross *et al.* 1992), implying that, even within a single cell, the responsiveness to Ins(1,4,5)P_3 may be regulated by a heterogeneous population of receptors. Whether heterogeneity can exist between the subunits in a single Ins(1,4,5)P_3 receptor remains to be determined, but this would provide the basis for further diversity reminiscent of many cell surface ligand-gated ion channels (Unwin, 1989).

Ins(1,4,5)P_3 receptor regulation

Ins(1,4,5)P_3 receptors are the targets of a number of allosteric regulators, including protein kinases, adenine nucleotides, pH and divalent cations, all of which may play a part in determining the responsiveness of cells to Ins(1,4,5)P_3. Further complexity is now apparent with the discovery that different Ins(1,4,5)P_3 receptor subtypes and splice variants show different patterns of allosteric regulation.

Phosphorylation by cyclic-AMP-dependent protein kinase

Cross-talk between the cyclic AMP and phosphoinositide signalling pathways is well documented (Berridge, 1975; Jenkinson and Koller, 1977; Rasmussen and Barrett, 1984).

In hepatocytes, for example, the frequency of Ca^{2+} spikes triggered by hormones linked to Ins(1,4,5)P_3 production is increased by activation of receptors coupled to adenylate cyclase. Cyclic AMP is now known to phosphorylate Ins(1,4,5)P_3 receptors *via* the activation of PKA (Walaas *et al.* 1986). Two putative consensus sequences for phosphorylation by PKA (X-Arg-Arg-X-Ser-X) have been identified in the coupling domain of both the type 1 (Furuichi *et al.* 1989; Mignery *et al.* 1990) and type 2 (Südhof *et al.* 1991) Ins(1,4,5)P_3 receptors, fuelling speculation that this region of the receptor is the major target for allosteric regulators. A third putative consensus sequence located in the Ca^{2+} channel domain has also been reported (Mignery *et al.* 1990). Although these consensus sequences are often conserved between tissues, phosphorylation of Ins(1,4,5)P_3 receptors by PKA appears to have different effects in different cell types. Cerebellar microsomes, for example, are 10-fold less sensitive to Ins(1,4,5)P_3 following cyclic-AMP-dependent phosphorylation (Supattapone *et al.* 1988*a*), whereas Ca^{2+} release from permeabilized hepatocytes is potentiated by pretreatment of the cells with the catalytic subunit of PKA (Burgess *et al.* 1991). In platelets, PKA also phosphorylates Ins(1,4,5)P_3 receptors but there are conflicting reports as to the effects of PKA on Ca^{2+} release (Enouf *et al.* 1987; Quinton and Dean, 1992). PKA markedly stimulates Ca^{2+} uptake into the platelet dense tubular system, whereas the reported effects of PKA on Ins(1,4,5)P_3-stimulated Ca^{2+} release are modest by comparison (Enouf *et al.* 1987; Quinton and Dean, 1992) and may be confused with regulatory effects of luminal Ca^{2+} on the Ins(1,4,5)P_3 receptors (see below).

The tissue-specific effects of PKA on Ins(1,4,5)P_3-stimulated Ca^{2+} fluxes may arise from differences in the phosphorylation patterns of distinct Ins(1,4,5)P_3 receptor subtypes and splice variants (see above). For example, while the long, neuronal form of the type 1 Ins(1,4,5)P_3 receptor is phosphorylated on both PKA consensus sequences in the coupling domain (Ser-1589 and Ser-1756), the shorter splice variant, localized in peripheral tissues, is phosphorylated at lower substrate concentrations and almost exclusively on Ser-1589 (Danoff *et al.* 1991). Differences in patterns of phosphorylation also exist between species: the recently cloned *Drosophila* Ins(1,4,5)P_3 receptor, for example, appears not to contain potential PKA phosphorylation sites (Yoshikawa *et al.* 1992).

Phosphorylation by protein kinase C and Ca^{2+} calmodulin-dependent protein kinase II

In addition to PKA, Ins(1,4,5)P_3 receptors are now known to be targets for phosphorylation by protein kinase C (PKC) and Ca^{2+} calmodulin-dependent protein kinase II (CaM kinase II) (Yamamoto *et al.* 1989; Ferris *et al.* 1991). The three enzymes phosphorylate the receptor at different sites (Ferris *et al.* 1991), suggesting that each may have a specific and independent role in controlling Ins(1,4,5)P_3 receptor activity. In each case, phosphorylation is reported to be stoichiometric (Supattapone *et al.* 1988*a*; Ferris *et al.* 1991), with each subunit of a receptor being phosphorylated at a single site by each kinase. However, in the case of phosphorylation by PKA, this may be an oversimplification since Ins(1,4,5)P_3 receptors are known to show distinctive patterns of phosphorylation depending on the expression of various alternatively spliced sequences (Danoff *et al.* 1991; see above).

At present, the functional significance of these phosphorylation events for Ins(1,4,5)P_3 receptor activity is poorly understood. PKC-mediated phosphorylation of Ins(1,4,5)P_3 receptors is reported to accelerate Ca^{2+} release (Matter *et al.* 1993), whereas the effects of phosphorylation by PKA may be either stimulatory or inhibitory (as discussed above). It is clear that phosphorylation of Ins(1,4,5)P_3 receptors provides a means by which different limbs of the intracellular signalling pathways may overlap to regulate tightly the release of intracellular Ca^{2+} stores. The recent finding that purified Ins(1,4,5)P_3 receptors themselves have intrinsic protein kinase activity, such that they are able both to autophosphorylate and to phosphorylate exogenous substrates (Ferris *et al.* 1992), further suggests that phosphorylation of the receptor is an important mechanism for regulating the actions of Ins(1,4,5)P_3.

Adenine nucleotides

ATP regulates the activity of Ins(1,4,5)P_3 receptors in a biphasic manner. Small elevations in ATP concentration (in the range 10–500 $\mu mol\,l^{-1}$) increase the binding of Ins(1,4,5)P_3 to its receptor (Spät *et al.* 1992) and potentiate Ins(1,4,5)P_3-stimulated Ca^{2+} fluxes (Smith *et al.* 1985; Suematsu *et al.* 1985; Ferris *et al.* 1990; Iino, 1991), whereas at higher concentrations (>500 $\mu mol\,l^{-1}$) ATP inhibits both Ins(1,4,5)P_3 binding (Guillemette *et al.* 1987; Willcocks *et al.* 1987; Nunn and Taylor, 1990) and Ca^{2+} release (Ferris *et al.* 1990; Iino, 1991).

The stimulatory effects of ATP are mimicked by other adenine nucleotides (Ferris *et al.* 1990; Iino, 1991) but not by GTP (Ferris *et al.* 1990), suggesting that the adenine base is important in mediating these effects. Electrophysiological studies of purified Ins(1,4,5)P_3 receptors reconstituted into planar lipid bilayers have demonstrated that ATP acts directly on the Ins(1,4,5)P_3 receptor to promote Ca^{2+} release either by increasing the open probability of Ca^{2+} channels (Ehrlich and Watras, 1988) or by promoting the formation of a larger-conductance Ca^{2+} channel (Maeda *et al.* 1991). Two putative adenine-nucleotide-binding sites (Gly-X-Gly-X-X-Gly) have been identified in the coupling domain of the cerebellar Ins(1,4,5)P_3 receptor (Furuichi *et al.* 1989; Mignery *et al.* 1990), and a third site is present in non-neuronal tissues following the deletion of the SII splice sequence. Binding of ATP to these sites is thought to mediate the stimulatory effects of ATP on the Ins(1,4,5)P_3 receptor (Ferris *et al.* 1990; Maeda *et al.* 1991).

The inhibitory effect of ATP arises from competition between ATP and Ins(1,4,5)P_3 for the Ins(1,4,5)P_3-binding site (Willcocks *et al.* 1987; Nunn and Taylor, 1990; Iino, 1991). ATP-mediated inhibition of [^{3}H]Ins(1,4,5)P_3 binding to purified receptors is mimicked by GTP and pyrophosphate (Maeda *et al.* 1991), suggesting that it is the binding of the pyrophosphate group of ATP to the Ins(1,4,5)P_3 receptor that is important in mediating the inhibitory effects of ATP.

Although ATP levels in the cytosol may be important in setting the sensitivity of Ins(1,4,5)P_3 receptors, there is still doubt as to whether Ins(1,4,5)P_3 receptors respond to changes in ATP concentration following agonist stimulation. From nuclear magnetic resonance studies in smooth muscle bundles, the intracellular ATP concentration has been shown to remain fairly constant, in the range 1–2 $mmol\,l^{-1}$, even following maximal agonist stimulation (Kushmeric *et al.* 1986). It is still conceivable, however, that local

changes in ATP concentration, possibly in the vicinity of the Ca^{2+}-ATPase pumps, which may be maximally activated following cell stimulation, may be important in controlling the activity of Ins(1,4,5)P_3 receptors in intact cells.

pH

Binding of Ins(1,4,5)P_3 to its receptor and Ins(1,4,5)P_3-stimulated Ca^{2+} release are both favoured by alkaline pH, optimally in the range 7.5–9 (Brass and Joseph, 1985; Worley *et al.* 1987; Joseph *et al.* 1989). These effects are mediated by an increase in the affinity of Ins(1,4,5)P_3 receptors for their ligand rather than by an increase in the number of available Ins(1,4,5)P_3-binding sites (Joseph *et al.* 1989).

The steep dependence of Ins(1,4,5)P_3 binding on pH suggests that small increases in intracellular pH may have a stimulatory effect on Ca^{2+} release in an intact cell. Indeed, growth factors and hormones that stimulate phosphoinositide turnover are also known to cause alkalization of the cytosol (by up to 0.3pHunits) *via* the activation of protein kinase C (PKC) (Horne *et al.* 1981). Once activated, PKC is able to stimulate the membrane-bound Na^+/H^+ antiporter, which extrudes H^+ from the cytosol. This raises the attractive possibility of crosstalk between the two limbs of the phosphoinositide signalling pathway.

Sulphydryl reagents

In a number of cell types, sulphydryl reagents such as thimerosal and oxidized glutathione trigger Ca^{2+} oscillations (Swann, 1991; Bootman *et al.* 1992). In hamster eggs, these oscillations can be inhibited by monoclonal antibodies directed against the C terminus of the Ins(1,4,5)P_3 receptor, suggesting a central role for Ins(1,4,5)P_3 receptors in the responses (Miyazaki *et al.* 1992). It has been proposed that the stimulation of Ca^{2+} oscillations by oxidized glutathione and thimerosal is dependent on their ability to increase the affinity of Ins(1,4,5)P_3 receptors for Ins(1,4,5)P_3 (Hilly *et al.* 1993) and subsequently to sensitize the release of Ins(1,4,5)P_3-sensitive Ca^{2+} stores (Missiaen *et al.* 1991), although several groups have reported inhibitory effects of thimerosal on Ins(1,4,5)P_3 receptors (Adunyah and Dean, 1986; Guillemette and Segui, 1988). These findings are reconciled by the observation that, at low concentrations, thimerosal sensitizes Ins(1,4,5)P_3-stimulated Ca^{2+} release from cerebellar microsomes, but at higher concentrations it is inhibitory (Sayers *et al.* 1993).

In resting cells, the concentration of oxidized glutathione is more than 20-fold lower than the concentration of the reduced form, and is out of the range at which it is believed to regulate the activity of Ins(1,4,5)P_3 receptors. We do not yet know whether physiological changes in redox state within cells can influence the activity of Ins(1,4,5)P_3 receptors.

Cytosolic Ca^{2+}

The regulatory effects of cytosolic Ca^{2+} on the Ins(1,4,5)P_3 receptor are confusing. Ca^{2+} inhibits [^{3}H]Ins(1,4,5)P_3 binding to cerebellar membranes (Worley *et al.* 1987), stimulates binding in liver (Pietri *et al.* 1990), but has no effect on the binding of Ins(1,4,5)P_3 to its receptor in vas deferens (Mourey *et al.* 1990). Similarly, Ca^{2+}

reportedly inhibits Ca^{2+} release in some instances (Joseph *et al.* 1989; Willems *et al.* 1990; Zhao and Muallem, 1990) but not in others (Brass and Joseph, 1985). While some tissue specificity may exist, other factors have certainly contributed to these inconsistent observations. The inhibitory effect of Ca^{2+} on cerebellar Ins(1,4,5)P_3 receptors has been ascribed to an accessory protein, termed calmedin, which is enriched in cerebellum but exists at much lower concentrations in the periphery (Danoff *et al.* 1988). Once purified, the cerebellar Ins(1,4,5)P_3 receptor is insensitive to Ca^{2+}, but sensitivity is restored by addition of solubilized cerebellar membranes enriched in calmedin activity (Supattapone *et al.* 1988*b*). However, recent evidence suggests that rather than being a Ca^{2+}-binding accessory protein, calmedin may be a Ca^{2+}-sensitive isoform of PLC (Mignery *et al.* 1992). Apparent Ca^{2+}-dependent inhibition of Ins(1,4,5)P_3 binding might then simply reflect Ca^{2+}-stimulated production of endogenous Ins(1,4,5)P_3, which would compete with radiolabelled Ins(1,4,5)P_3 for the receptor. Another complexity arises from the observation that, at high concentrations, a number of frequently used Ca^{2+} buffers, including the fluorescent Ca^{2+} indicator dye Fura-2, have been shown to act as competitive antagonists at the Ins(1,4,5)P_3 receptor (Richardson and Taylor, 1993). As free Ca^{2+} concentrations are adjusted in experimental protocols, so too are the concentrations of free Ca^{2+} chelators.

Finally, regulation of Ins(1,4,5)P_3 receptors by cytosolic Ca^{2+} is now known to be highly concentration-dependent. Small elevations in cytosolic [Ca^{2+}] (<300nmoll^{-1}) potentiate both Ca^{2+} release and channel opening, whereas at higher concentrations Ca^{2+} is inhibitory (Iino, 1990; Bezprozvanny *et al.* 1991; Finch *et al.* 1991). These effects of Ca^{2+} operate on a rapid time-scale, suggesting that they may play an important role in the regulation of Ins(1,4,5)P_3-stimulated Ca^{2+} release in intact cells (Iino and Endo, 1992). In liver, at least three distinct affinity and conductance states of the Ins(1,4,5)P_3 receptor have been identified (Fig. 3). When cytosolic Ca^{2+} is elevated (<700nmoll^{-1}), receptors are converted from a state with low affinity for Ins(1,4,5)P_3 to a state with high affinity (Pietri *et al.* 1990), both states being able to conduct Ca^{2+} in response to Ins(1,4,5)P_3 (Marshall and Taylor, 1993). As Ca^{2+} is further elevated (>1 μmoll^{-1}), receptors maintain their high-affinity conformation but are unable to conduct Ca^{2+} when bound to Ins(1,4,5)P_3.

Considering the oscillatory changes in cytosolic Ca^{2+} during cell activation, Ca^{2+} itself has the potential to regulate it own release from Ins(1,4,5)P_3-sensitive Ca^{2+} stores. Such regulatory control is, therefore, a key component in several recent models used to describe the complex organization of Ca^{2+} signals (discussed below).

Luminal Ca^{2+}

Ca^{2+} within the lumen of the sarcoplasmic reticulum has been shown to enhance opening of ryanodine receptors in skeletal muscle (Nelson and Nelson, 1990), prompting speculation of a similar role for luminal Ca^{2+} in regulating Ins(1,4,5)P_3 receptors (Irvine, 1991). Evidence for such a role, however, has been difficult to obtain for a number of reasons. Unlike the situation in the cytosol, where Ca^{2+} buffers can be used to titrate the cytosolic [Ca^{2+}], the [Ca^{2+}] within the lumen of stores cannot be reliably measured. Experimental procedures have therefore relied on the manipulation of luminal [Ca^{2+}],

either by overloading the Ca^{2+} stores (Missiaen *et al.* 1991) or by progressively discharging full stores using Ca^{2+} ionophores or Ca^{2+} pump inhibitors (Nunn and Taylor, 1990; Marshall and Taylor, 1993). To compound these problems, the effects of luminal Ca^{2+} on Ins(1,4,5)P_3 receptor activity are modest compared with those of cytosolic Ca^{2+}. Depleting Ca^{2+} stores reduces their affinity for Ins(1,4,5)P_3 only twofold (Oldershaw and Taylor, 1993), with a similar effect on the EC_{50} for Ca^{2+} release (Nunn and Taylor, 1990). The failure of some groups to detect effects of luminal Ca^{2+} on Ins(1,4,5)P_3 receptors (Shuttleworth, 1992; Combettes *et al.* 1993) may be due to the extensive store depletion that is required before these effects become evident (Marshall and Taylor, 1993).

Although the effects of luminal Ca^{2+} on the Ins(1,4,5)P_3 receptor are modest, there is some evidence to suggest that changes in luminal [Ca^{2+}] also modulate the regulatory

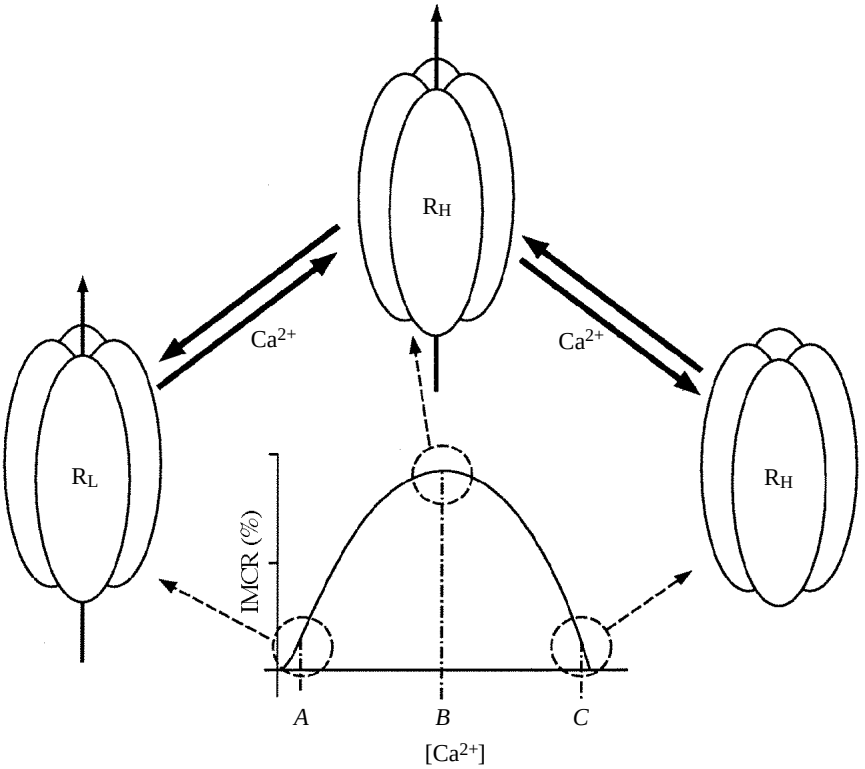

Fig. 3. Interconversion between different conformations of the liver Ins(1,4,5)P_3 receptor by cytosolic Ca^{2+}. Ins(1,4,5)P_3-mediated Ca^{2+} release (IMCR) is biphasically controlled by cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) (centre). Small elevations in $[Ca^{2+}]_i$ (<300nmol l^{-1} Ca^{2+}; *A* to *B*) increase the extent of IMCR, whereas larger elevations (>300nmol l^{-1} Ca^{2+}; *B* to *C*) reduce IMCR. These changes in IMCR reflect conversion between different conformations of the Ins(1,4,5)P_3 receptor. At low $[Ca^{2+}]_i$ (*A*), receptors have a low affinity for Ins(1,4,5)P_3 (R_L) and are able to conduct Ca^{2+}. Following small elevations in $[Ca^{2+}]_i$ (100–300nmol l^{-1}; *B*), receptors remain active and are shifted to a higher-affinity conformation (R_H). Further elevations in $[Ca^{2+}]_i$ (>1 μmol l^{-1}; *C*) convert receptors to a non-conducting conformation which maintains a high affinity for Ins(1,4,5)P_3.

effects of cytosolic Ca^{2+} (Missiaen *et al.* 1992): thus, a three-way balance may exist in which Ins(1,4,5)P_3, cytosolic Ca^{2+} and luminal Ca^{2+} interdependently regulate the opening of Ins(1,4,5)P_3 receptors.

Mg^{2+}

Mg^{2+} inhibits both Ins(1,4,5)P_3 binding (Varney *et al.* 1990; White *et al.* 1991) and Ins(1,4,5)P_3-stimulated Ca^{2+} mobilization (Volpe *et al.* 1990) at concentrations slightly lower than those found in intact cells (Brooks and Bachelard, 1989). At present, we have little information concerning Mg^{2+} concentration fluctuations during agonist stimulation, but it is possible that, when cytosolic [Ca^{2+}] is increased, Mg^{2+} may be displaced from Ca^{2+}/Mg^{2+}-binding proteins, such as parvalbumin, thereby transiently elevating intracellular Mg^{2+} levels. Interpreting how Mg^{2+} is affecting Ins(1,4,5)P_3 receptors may be more difficult: apart from its putative role as an allosteric regulator, Mg^{2+} also stimulates metabolism of Ins(1,4,5)P_3 *via* activation of the 5-phosphatase and is essential for Ins(1,4,5)P_3 receptor autophosphorylation (Ferris *et al.* 1992).

Ins(1,4,5)P_3-stimulated Ca^{2+} mobilization

When Ins(1,4,5)P_3 binds to its receptor, there is a conformational change in the receptor complex (Mignery and Südhof, 1990) and an increase in the frequency of Ca^{2+} channel opening (Ehrlich and Watras, 1988), resulting in the release of Ca^{2+} from intracellular stores (Streb *et al.* 1983). Rapid kinetic measurements of Ca^{2+} release in permeabilized (Champeil *et al.* 1989; Meyer *et al.* 1990) and intact (Ogden *et al.* 1990) cells have revealed a delay of less than 100ms between the addition of Ins(1,4,5)P_3 and the onset of Ca^{2+} mobilization, indicating that the latency of receptor-mediated [Ca^{2+}] changes is primarily determined by the rate of Ins(1,4,5)P_3 formation.

Electrophysiological studies suggest that four subconductance states exist for single Ins(1,4,5)P_3 receptors (Watras *et al.* 1991); these may arise from different numbers of Ins(1,4,5)P_3 molecules binding to the tetrameric receptor complex. With the possibility that heterogeneous Ins(1,4,5)P_3 receptor subunits may exist in a single receptor, there is certainly scope for a large family of Ins(1,4,5)P_3 receptors that differ in their Ca^{2+} conductances. Some groups have reported cooperative Ins(1,4,5)P_3-stimulated Ca^{2+} release from intracellular stores (Champeil *et al.* 1989; Meyer *et al.* 1990). However, such cooperativity is not evident under conditions in which the cytosolic [Ca^{2+}] is clamped (Finch *et al.* 1991) and is, therefore, more likely to be a consequence of the stimulatory effects of cytosolic Ca^{2+} on Ins(1,4,5)P_3-stimulated Ca^{2+} release (see above). Ca^{2+} release in intact cells may, nevertheless, show steep dependence on Ins(1,4,5)P_3 concentrations as a result of this regulatory effect of cytosolic Ca^{2+}.

A further complexity in the mechanism of Ins(1,4,5)P_3-stimulated Ca^{2+} release is the phenomenon of ‘quantal’ Ca^{2+} mobilization, whereby low concentrations of Ins(1,4,5)P_3 are able to mobilize only a fraction of the Ins(1,4,5)P_3-sensitive Ca^{2+} stores (Muallem *et al.* 1989; Taylor and Potter, 1990), despite the inability of Ins(1,4,5)P_3 receptors to desensitize intrinsically (Oldershaw *et al.* 1992). Although a number of explanations for this phenomenon have been proposed, neither the mechanism nor the physiological significance of quantal Ca^{2+} mobilization is understood.

Role of Ins(1,4,5)P_3 in the organization of Ca^{2+} signals

The Ca^{2+} signals evoked by receptor activation are complex, occurring in many cells as repetitive Ca^{2+} spikes (see Berridge, 1990), which may be arranged spatially as regenerative waves (Jacob, 1990; Rooney *et al.* 1990) or even as spirals of Ca^{2+} release (Lechleiter *et al.* 1991). Models which describe the generation and propagation of Ca^{2+} signals are plentiful (reviewed by Berridge, 1990) and differ in the extent to which Ins(1,4,5)P_3 contributes. Ins(1,4,5)P_3 plays an important role in triggering regenerative Ca^{2+} signals in many cells (Oron *et al.* 1985; Parker and Miledi, 1986; Wakui *et al.* 1989), and more recently it has been shown to be essential for the propagation of Ca^{2+} waves in *Xenopus* oocytes (DeLisle and Welsh, 1992) and fertilized hamster eggs (Miyazaki *et al.* 1992). Consistent with these observations, recent models to describe how Ca^{2+} waves propagate across cells invoke the sequential release of Ins(1,4,5)P_3-sensitive Ca^{2+} stores: it is necessary, therefore, to determine how these stores are regulated so as to release their Ca^{2+} in a sequential manner.

If diffusion of Ins(1,4,5)P_3 *per se* were to account for the sequential discharge of Ca^{2+} stores, one would expect Ca^{2+} waves to propagate across cells at a non-uniform rate, a feature not easily reconciled with experimental observations (Rooney *et al.* 1990).

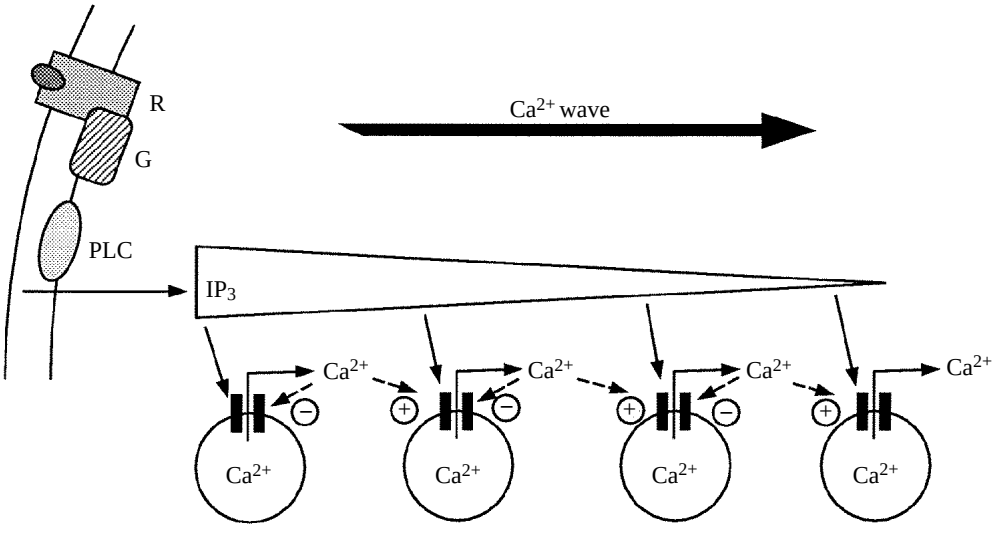

Fig. 4. Model for Ca^{2+} wave propagation. Stimulation of G-protein-linked (G) cell surface receptors (R) leads to the activation of phospholipase C (PLC) and the production of Ins(1,4,5)P_3 (IP_3). Ca^{2+} stores closest to the site of Ins(1,4,5)P_3 production release their Ca^{2+} in response to the high concentration of Ins(1,4,5)P_3. The Ca^{2+} released from these stores sensitizes Ins(1,4,5)P_3 receptors further from the site of Ins(1,4,5)P_3 production, allowing them to respond to the lower concentration of Ins(1,4,5)P_3. In this way, the Ca^{2+} wave propagates by the sequential discharge of Ins(1,4,5)P_3-sensitive Ca^{2+} stores. Once Ca^{2+} has been released from a store, the Ins(1,4,5)P_3 receptors on that store are exposed to a much higher [Ca^{2+}] and become desensitized, allowing Ca^{2+} to be pumped back into the stores in preparation for a subsequent Ca^{2+} wave.

Another possibility is that release of Ins(1,4,5)P_3-sensitive Ca^{2+} stores is regulated by a signal transmitted concomitantly with the Ca^{2+} wave; the complex control of Ins(1,4,5)P_3 receptor sensitivity by cytosolic Ca^{2+} (discussed above) suggests that Ca^{2+} itself is a likely candidate. The Ca^{2+} wave might then propagate across the cell as follows (Fig. 4). Prior to receptor activation, resting cytosolic [Ca^{2+}] is low (approximately 100nmol l^{-1}), so that Ins(1,4,5)P_3 receptors are in a conformation with low affinity for Ins(1,4,5)P_3 (see Fig. 3). Activation of cell surface receptors triggers production of Ins(1,4,5)P_3 at the plasma membrane, which stimulates neighbouring Ins(1,4,5)P_3-sensitive stores to release their Ca^{2+} into the cytosol. The Ca^{2+} released from these stores then sensitizes Ins(1,4,5)P_3 receptors on stores deeper inside the cell, allowing them to respond to the lower levels of Ins(1,4,5)P_3 further from the plasma membrane. The Ca^{2+} wave would then propagate by the concerted actions of Ins(1,4,5)P_3 and Ca^{2+} on Ins(1,4,5)P_3 receptors. Once a store releases its Ca^{2+}, Ins(1,4,5)P_3 receptors on that store may become inactive as a result of the inhibitory effects of the high local cytosolic [Ca^{2+}] and the reduced luminal [Ca^{2+}] (discussed above). This would render Ins(1,4,5)P_3-sensitive Ca^{2+} stores refractory to Ins(1,4,5)P_3, allowing them to refill in preparation for a subsequent Ca^{2+} wave. Support for this model comes from the study of agonist-evoked oscillations in pancreatic acinar cells, where the frequency of oscillations is increased in response to small elevations in cytosolic Ca^{2+} (<200nmol l^{-1}), but reduced when cytosolic Ca^{2+} is raised above 500nmol l^{-1} (Zhang *et al.* 1992).

Recently, single spontaneous Ca^{2+} spikes have been recorded in populations of permeabilized hepatocytes (Missiaen *et al.* 1991, 1992); they have been attributed to the synchronized discharge of Ins(1,4,5)P_3-sensitive Ca^{2+} stores. Overloading of these stores with Ca^{2+} appeared to sensitize Ins(1,4,5)P_3-stimulated Ca^{2+} release, allowing the stores to respond to basal levels of Ins(1,4,5)P_3. These responses were preceded by a pacemaker rise in cytosolic Ca^{2+} concentration, which may reflect the involvement of a positive feedback effect of cytosolic [Ca^{2+}] on Ins(1,4,5)P_3-stimulated Ca^{2+} release during the rising phase of the spikes. Interestingly, these spontaneous Ca^{2+} spikes in permeabilized cell suspensions are critically dependent on ATP concentration, pH, cytosolic [Ca^{2+}] and cell density, and are sensitized by sulphydryl reagents (Missiaen *et al.* 1992). An attractive possibility is that they represent a crude demonstration *in vitro* of the Ca^{2+} spikes seen in intact cells: here, the responses are evident in a system in which the cytosol from a population of cells has been effectively pooled. If this proves to be the case, it is clear that a number of cytosolic regulators of Ins(1,4,5)P_3 receptor activity are critically important in setting the conditions for the generation of Ca^{2+} signals.

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