REVIEW**Structure and Function of Inositol 1,4,5-Trisphosphate Receptor**

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ABSTRACT—Generation of intracellular Ca^{2+} signals in response to Ca^{2+} -mobilizing stimuli is a critical event in the control of many cellular processes. Inositol 1,4,5-trisphosphate (IP_3) represents a dominant second messenger subserving the release of Ca^{2+} from intracellular store sites. The protein on the surface of which the IP_3 receptor is located comprises an IP_3 -gated Ca^{2+} channel, and binding of IP_3 to this receptor triggers the release of Ca^{2+} through this channel. The receptor for IP_3 displays a close resemblance to the ryanodine receptor, another intracellular Ca^{2+} channel, in many molecular and physiological properties. Many lines of evidence strongly suggest the central role that the IP_3 receptor plays in the conversion of numerous external stimuli to intracellular Ca^{2+} signals characterized by complex spatiotemporal patterns such as Ca^{2+} waves and oscillations. In this review, we shall summarize our current knowledge of the structure and function of the IP_3 receptor in order to understand the way how the activity of this important receptor is regulated to accomodate itself to the generation of diverse intracellular Ca^{2+} signals.

Keywords: Inositol 1,4,5-trisphosphate (IP_3), IP_3 receptor, Ryanodine receptor, Ca^{2+} -mobilization, Phospholipase C

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I. Introduction

Intracellular calcium plays a crucial role in the control of many cellular processes as diverse as cell motility, contraction, secretion, cell proliferation, sensory perception and neuronal signaling (1–4). Generation of intracellular

Ca^{2+} signals with complex spatiotemporal patterns is dynamically and precisely controlled by both the entry of external Ca^{2+} and the release of internal Ca^{2+} . In response to many stimuli such as neurotransmitters, hormones and growth factors, phosphatidylinositol 4,5-bisphosphate (PIP_2) in the plasma membrane is hydrolyzed by phospholipase C (PLC) to produce inositol

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1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG). IP₃ plays a dominant role as a second messenger molecule for the release of Ca²⁺ from intracellular stores, while DAG activates protein kinase C (PKC). It binds to an intracellular receptor (IP₃ receptor) located on the surface of intracellular Ca²⁺ stores. The IP₃ receptor is at the same time a release channel for luminal Ca²⁺ whose molecular and physiological properties closely resemble those of another intracellular, Ca²⁺-mobilizing channel, i.e., the ryanodine receptor. Ca²⁺ release occurs upon binding of IP₃ to the receptor. Thus, the IP₃ receptor plays a central role in conversion of IP₃ signals produced by external stimuli to intracellular Ca²⁺ signals. Many lines of evidence now indicate that regenerative, intricately regulated Ca²⁺ release through these intracellular Ca²⁺ channels is mainly responsible for generation of intracellular Ca²⁺ signals with complex spatiotemporal patterns such as calcium waves and oscillations. In this review, we shall summarize the current understanding of the structure and function of the IP₃ receptor to understand the way how the activity of the IP₃ receptor is regulated to accommodate itself to generation of diverse intracellular Ca²⁺ signals. Several reviews (5–10) can be consulted for information not focused on in this article.

II. Formation of IP₃

Formation of IP₃ is prerequisite for both the release of Ca²⁺ from intracellular stores as well as the entry of external Ca²⁺. There are two major pathways for hydrolysis of PIP₂ to IP₃: one is initiated by G protein-linked receptors characterized by the presence of seven transmembrane-spanning domains in their molecules and the other by tyrosine kinase-linked receptors characterized by a single transmembrane-spanning domain (Table 1). The G protein-linked receptors stimulate PLC β isozymes, while the tyrosine kinase-linked receptors stimulate PLC γ iso-

zymes to convert PIP₂ to IP₃ and DAG. Both pertussis toxin-sensitive and -insensitive heterotrimeric G proteins link the activation of receptors with the stimulation of PLC β (11). Upon activation, the heterotrimeric G proteins dissociate into G α and G $\beta\gamma$ subunits, both of which stimulate different PLC β isozymes (12). The involvement of pertussis toxin-insensitive G α_q and other members of the family, G α_{11} , G α_{14} and G α_{16} , in the stimulation of PLC β is now well-established. The pertussis toxin-sensitive G proteins that mediate PLC stimulation are less well-characterized, although the involvement of G α_o and G α_i subunits has been suggested (13). Stimulation of PLC β by G $\beta\gamma$ subunits is now firmly established (12). G α and G $\beta\gamma$ subunits appear to interact with distinct sites of the PLC β molecule, implicating independent regulation by both subunits of the heterotrimeric G protein.

Tyrosine kinase receptors such as receptors for platelet-derived growth factor and epidermal growth factor (Table 1) generate IP₃ by direct stimulation of PLC γ . These receptor molecules contain cytoplasmic tyrosine kinase domains. Upon ligand binding, the receptor dimerizes and autophosphorylates each other on specific tyrosine residues that form the docking sites responsible for interaction with the SH2 domains of PLC γ . Interaction of PLC γ with the specific phosphotyrosine residues of receptors brings about phosphorylation of the PLC γ on at least 3 tyrosine residues and leads to stimulation of the enzyme and production of IP₃ and DAG (2). A similar PLC γ stimulation process operates in the activation of IgM receptors in B lymphocytes and the activation of T-cell antigen receptors. These receptors lack tyrosine kinase domains but they recruit cytoplasmic *src* family tyrosine kinases such as *fyn* and *lck* (14). In general, formation of IP₃ in the activation of tyrosine kinase-linked receptors is slower and the resulting Ca²⁺ signal is smaller though of longer duration than that due to the activation of G protein-linked receptors (4, 15).

Table 1. Cell surface receptors^a that increase inositol 1,4,5-trisphosphate (IP₃)

G protein-linked receptors Via PLC β	Tyrosine kinase-linked receptors Via PLC γ
Adrenergic: α_{1A} , α_{1B} , α_{1D}	Epidermal growth factor receptor
Muscarinic: M ₁ , M ₃ , M ₅	Platelet-derived growth factor receptor
Angiotensin: AT ₁	Fibroblast growth factor receptor
Bombesin: BB ₁ , BB ₂	ErbB2
Bradykinin: B ₁ , B ₂	T cell receptor
Cholecystokinin: CCK _A , CCK _B	
Endothelin: ET _A , ET _B	
Glutamate (EAA): mGlu ₁ , mGlu ₅	
Histamine: H ₁	
5-HT: 5-HT _{2A} , 5-HT _{2B} , 5-HT _{2C}	
Leukotriene: BLT, CysLT ₁	
Melatonin: ML ₂	
Oxytocin: OT	
Prostanoid: FP, TP, EP ₁ , EP ₃	
Purinergic: P _{2U} , P _{2Y}	
Tachykinin: NK ₁ , NK ₂ , NK ₃	
Vasopressin: V _{1A} , V _{1B}	

^aReceptor nomenclature was based on that used in TIPS Receptor and Ion Channel Nomenclature Supplement 1996, Elsevier Trends Journals, Cambridge (1996), which was compiled in association with the IUPHAR.

The activation pathway for PLC δ , one of the three major PLC families (β , γ and δ), is not known. The enzyme contains one EF-hand motif and may represent a calcium-sensitive form of PLC. The PLC δ_1 activity is reported to be increased in the aorta of spontaneously hypertensive rats (16). Recently, a high affinity binding of IP₃ to the PLC δ_1 isozyme was demonstrated (17). The phospholipase activity of the isozyme was strongly inhibited upon binding of IP₃.

III. Structure and expression of IP₃ receptor

1. Subtypes

To date, IP₃ receptor has been purified to apparent homogeneity from cerebellum (18, 19), smooth muscle (20–22) and platelet (23). The purified IP₃ receptors are glycosylated proteins with molecular masses ranging from 220 to 260 kDa on SDS-PAGE. The Purkinje cells in the cerebellum are a very rich source of the receptor, and hence our current knowledge of the molecular properties of the IP₃ receptor originated mostly from studies with the cerebellar receptor. Molecular cloning studies revealed the existence of at least three types of mammalian IP₃-receptor subunits encoded by different genes designated as type 1 (24–26), type 2 (27, 28) and type 3 (28–30). Putative type 4 and 5 receptors, which are highly homologous to the type 2 receptor, have also been sequenced partially (31, 32).

Among these subtypes, type 1 IP₃ receptor, the representative cerebellar receptor, is ubiquitously expressed in a wide variety of tissues and is best characterized now. As illustrated in Fig. 1, the type 1 IP₃ receptor is composed of 2,749 amino acids with a calculated Mr of 313 K (an S1⁺/S2⁺ splicing variant, see below) in the mouse (24) and rat (25). It can be structurally and functionally divided into three major domains: a ligand-binding domain in the NH₂-terminal portion (about 24% of the receptor molecule), a coupling or modulatory domain in the middle portion (about 60%) that transduces the signal of ligand binding, and a Ca²⁺ channel domain in the COOH-terminal portion (33). The type 2 IP₃ receptor is composed of 2,701 amino acids with a calculated Mr of 307 K in the rat (27) and shares 68% sequence identity with the type 1 receptor, while the type 3 IP₃ receptor is composed of 2,670 amino acids with a calculated Mr of 304 K in the rat (29) and shares 62% and 64% sequence identity with the type 1 and type 2 receptors, respectively. The overall structural organization is similar for all the three types of receptors. The percent identity among the three subtypes is 68% in the ligand-binding domain, 53% in the coupling domain, and 59% in the Ca²⁺ channel domain (29). The ligand-binding domain is the most conserved region, whereas the coupling domain separating

the ligand binding domain from the Ca²⁺ channel domain is the least conserved. The fact that the conservation is poor in the coupling domain suggests the possible operation of different types of regulation at this region among the three types. Alignment of the sequences of the three types of IP₃ receptor reveals a scattered and patchy distribution of conserved and variable regions (29, 30) as illustrated in Fig. 1. The ryanodine receptor, another intracellular Ca²⁺-release channel, is almost twice as large as the IP₃ receptor; nevertheless, it has fragmental sequence homology with the IP₃ receptor. Interestingly, the fragmental homology is concentrated in the ligand-binding domain and in the Ca²⁺ channel domain, implying their fundamental roles in the activity of the intracellular Ca²⁺-release channel (34).

The type 1 IP₃ receptor is further diversified by alternative splicing (35, 36). Two segments of alternative splicing are known in the receptor: one termed S1 is located in the NH₂-terminal ligand-binding domain and codes for 15 amino acids, and the other termed S2 lies in the coupling domain and codes for 40 amino acids (35) as shown in Fig. 1. In the nervous system, the longer form containing S2 predominates, while the shorter form lacking S2 predominates in the peripheral tissues (35–37). Studies of the mouse receptor have suggested the existence of intermediately spliced forms, i.e., the ones with only portions of the splicing segments (35). However, a detailed study using the RNase protection assay and polymerase chain reaction (PCR) analysis of rat type 1 IP₃ receptor did not provide evidence for the existence of intermediately spliced forms (37), raising the possibility that the degree of alternative splicing of type 1 IP₃ receptor differs in the two very similar species. No alternative splicing was detected at the corresponding positions in either the type 2 or type 3 receptor (38).

2. Tissue distribution

The type 1 IP₃ receptor is the predominant subtype expressed throughout the brain at both the mRNA and protein levels (31, 38–40). The level of type 1 receptor in the Purkinje cell in the cerebellum is particularly high, implying its specialized role in the cell type (39). The type 1 IP₃ receptor mRNA is expressed in a neuron-specific manner and present in low to moderate levels in the olfactory tubercle, cerebral cortex, CA1 region of the hippocampus, caudate-putamen, molecular layer of the cerebellum and choroid plexus (39). The type 1 IP₃ receptor is also widely distributed in tissues other than neuronal tissues: it is present at relatively high levels in smooth muscle, including vascular smooth muscle and vas deferens, and platelets and contained at low levels in other tissues (38, 40). The S1 and S2 splicing variants of type 1 IP₃ receptor are shown to be expressed in a tissue-specific and

The type 2 and type 3 IP₃ receptors exhibit a more limited distribution, and their expressions are generally at lower levels than the type 1 receptor. PCR and immunoblotting studies indicate that the type 2 receptor is expressed at relatively higher levels in the liver, pancreas, lung, testis and spleen and expressed at much lower levels in the brain including cerebellum (38, 40). The type 3 receptor is expressed at relatively higher levels in the

pancreas, intestine, lung, kidney and brain (29, 38, 40). The localization of type 3 receptor in astrocytes in the central nervous system of the rat has been immunohistochemically demonstrated (43). In general, multiple subtypes of IP₃ receptor are co-expressed in clonal cell lines irrespective of their origin, although each cell line appears to have its own slightly different pattern of expression (32, 38, 40).

3. Subcellular localization

Subcellular localization of type 1 IP₃ receptor has been best studied in the cerebellar Purkinje cell (44–46). The receptor is abundantly detected in smooth endoplasmic reticulum (ER) with particular enrichment in stacks of flattened cisternae and subplasmalemmal cisternae. A smaller amount of the receptor is also detected in rough ER and outer nuclear membrane, but it does not appear to be present in mitochondria, Golgi complex and plasma membrane. These findings indicate the localization of IP₃ receptors to putative intracellular Ca²⁺ stores, a localization that is compatible with its functional role as an intracellular Ca²⁺ channel. The plurality of the receptor in the stacks of flattened ER in the Purkinje cells may explain the exceptionally high concentration of type 1 IP₃ receptor in the cell type. On the stacks, the receptors appear to be apposed on an individual stack facing other receptors on the adjacent stack in a head-to-head configuration forming perpendicular bridges between stacks (47). Although IP₃ receptors are widely distributed, only a few studies have been conducted so far regarding the subcellular localizations of IP₃ receptors in non-neural tissues due probably to their low expression levels (48–50). Immunoelectron microscopy of rat vas deferens smooth muscle cells revealed the preferential clustering of IP₃ receptor in peripheral smooth ER, where calsequestrin, a luminal Ca²⁺ binding protein, was also enriched (49). In mouse choroidal cells, IP₃ receptor immunoreactivity was localized on the surfaces of several endomembrane systems including clear vesicles, tubules and vesicular profiles of smooth ER, rough ER and a part of the nuclear envelope, as well as clusters of ribosomes in the cytoplasmic matrix (50). The heart is known to be the tissue where IP₃ receptors are rarely expressed. However, the type 1 IP₃ receptor has been recently immunolocalized in rat hearts to intercalated discs of ventricular myocytes, while in contrast, the ryanodine receptor was observed in coincidence with the triad junction at the I-bands (51). The localization of the IP₃ receptor to intercalated discs of ventricular and atrial myocytes was also confirmed by immunoelectron microscopy. These results suggest a possible role of IP₃ receptor in Ca²⁺ entry through intercalated discs or intercellular signaling between cardiomyocytes.

Evidence for close association of IP₃ receptor with the plasma membrane was provided from cell fractionation studies that demonstrated the co-purification of IP₃ receptors with plasma membrane markers (52–55). These IP₃ receptors appeared to be located in a subcompartment of ER that was tightly bound to the plasma membrane since the receptors could be dissociated by treatments that disrupt the cytoskeleton (53, 55). Thus, the receptor seems to interact indirectly with the plasma membrane via the cytoskeleton. Binding sites for ankyrin, an adaptor protein connecting integral plasma membrane proteins to the cytoskeleton, have been demonstrated in IP₃ receptors (56–58). The close association of IP₃ receptor with the plasma membrane conforms with the conformational coupling model for “capacitative calcium entry”, a theory according to which the entry of external Ca²⁺ is regulated by the state of filling of intracellular Ca²⁺ stores (59). In this model, IP₃ receptors sense the filling state of luminal Ca²⁺ and transmit the information through their large cytoplasmic heads directly to Ca²⁺ entry channels on the plasma membrane.

The localization of an IP₃ receptor-like protein in the plasma membrane of various cell types including endothelial cells, smooth muscle cells and keratinocytes was demonstrated by immunocytochemical and immunocytochemical techniques (48). Interestingly, the protein was confined to caveolae, smooth invaginations of the plasma membrane where the plasma membrane Ca²⁺-extrusion pump was also co-localized (60), implying that the caveolae is a site of specialization for Ca²⁺ entry and extrusion. The presence of an IP₃ receptor-like protein in the plasma membrane was also demonstrated in some tissues or cells such as T lymphocytes and olfactory cilia (61, 62). In these cells, the presence of an IP₃-gated Ca²⁺ channel in the plasma membrane was demonstrated (63). Increase in the expression of type 3 IP₃ receptor has been recently demonstrated during the apoptosis of lymphocytes (64). The induced receptor was localized to the plasma membrane. Antisense cDNA to type 3 IP₃ receptor blocked the apoptosis, whereas either the sense probe to type 3 IP₃ receptor or antisense probe to type 1 IP₃ receptor did not. These results suggest that the selective expression of type 3 IP₃ receptor and their localization to the plasma membrane cause enhanced Ca²⁺ entry, leading to apoptosis. Furthermore, overexpression of type 3 IP₃ receptor in *Xenopus* oocytes had no effect on IP₃-induced Ca²⁺ release but it markedly enhanced capacitative Ca²⁺ entry. The type 3 IP₃ receptor was found to be localized preferentially to subplasmalemmal cisternae (65). These findings suggest a functional association of type 3 IP₃ receptor with the entry of external Ca²⁺.

4. Molecular structure

The molecular architecture of IP₃ receptor is well-characterized for the type 1 receptor. As illustrated in Fig. 1, the type 1 IP₃ receptor is structurally and functionally divided into three parts: the NH₂-terminal ligand binding domain, the coupling or modulatory domain and the COOH-terminal Ca²⁺ channel domain (33). The ligand binding domain together with the coupling domain constitute a large cytoplasmic head amounting to about 83% of the molecule. Electron microscopic observations (19, 20), a cross-linking study (66), and sucrose density gradient experiments (44) have suggested that the IP₃ receptor subunit tetramerizes to form an IP₃-gated Ca²⁺ channel. The expression of multiple subtypes of IP₃ receptor subunit in individual cells raises the possibility that a heterotetrameric IP₃ receptor is formed besides a homotetrameric IP₃ receptor. Indeed, the existence of heterotetrameric IP₃ receptors has been demonstrated in cultured cells (67–69) and rat liver (67). Different IP₃ receptor subtypes with distinct regulatory and Ca²⁺-channel properties will endow these heterotetrameric receptors with further functional diversity.

IP₃ binding domain: IP₃ binds to a single binding site in the NH₂-terminal ligand binding domain of the type 1 IP₃ receptor subunit (18–23). Therefore, the tetrameric receptor complex can bind four IP₃ molecules. Scatchard analyses of the kinetics of IP₃ binding to the receptor indicate no co-operativity giving Hill coefficients of around 1. The binding is of high affinity with K_D values ranging 2 to 100 nM and is highly specific to IP₃ (D-myo-inositol 1,4,5-trisphosphate) among various inositol phosphates. The inositol phosphate specificity and affinity are essentially the same in membranes and purified receptors (18, 21). About 650 amino acids of the NH₂-terminus was originally assigned to the portion required for IP₃ binding by deletion experiments (70). A recent more detailed study (71) has revealed the presence of a “core” region for IP₃ binding in the N-terminal residues from 226 to 578 of the type 1 receptor, where three basic amino acids (Arg-265, Lys-508 and Arg-511) are conserved among all subtypes of IP₃ receptor, and are shown to be essential for the IP₃ binding by mutagenesis experiments. These findings predict a critical pH dependency of the IP₃ binding to the receptor. Indeed, the binding is known to be increased at alkaline pH with the optimum around 9 and reduced at acidic pH: the binding at pH 8.5–9 is two- to three-fold greater than that at pH 7.5 (18, 20, 21). The steep slope of the pH curve for IP₃ binding provides a basis for sensitive regulation of IP₃ action that can be seen in response to alterations of intracellular pH.

Heparin is a potent competitive antagonist of the IP₃ receptor. It inhibits IP₃ binding to purified receptors (18–23) as well as the release of Ca²⁺ from intracellular

stores (72, 73), and IP₃-gated Ca²⁺ channel activity (74). However, its usefulness as a pharmacological tool for studies on intact cells is hampered because it may also inhibit the formation of IP₃ and activate the ryanodine receptor (75). Adenophostin A and B are newly discovered, potent and metabolically resistant agonists of IP₃ receptors (76, 77). In spite of structural differences from IP₃, adenophostins activate Ca²⁺ release channels with a potency 10 to 100-fold greater than IP₃, and their actions are completely blocked by heparin.

Coupling domain: The coupling domain separating the ligand binding domain from the Ca²⁺ channel domain is supposed to serve as a target of regulatory signals for the IP₃-gated Ca²⁺ release channel as well as a functional domain to transduce the IP₃ binding signal to the Ca²⁺ channel (33). As shown in Fig. 1, the domain contains putative binding sites for various modulators of the channel such as ATP, Ca²⁺, calmodulin (CaM), FK506-binding protein 12 (FKBP12), and phosphorylation sites for several protein kinases including cAMP-dependent protein kinase (PKA), cGMP-dependent protein kinase (PKG), PKC and Ca²⁺/calmodulin-dependent protein kinase II (CaMKII). These structural features found in this domain predict a complex regulation of IP₃-induced opening of Ca²⁺ channels. Many intracellular signaling pathways converge on this domain. We will later focus on several related mechanisms involved in the regulation of IP₃ receptor. As noted above, the homology among different IP₃ receptor subtypes is the lowest in the coupling domain, suggesting the operation of differential regulation in different subtypes.

Ca²⁺ channel domain: The hydropathy profile of the amino acid sequence of the Ca²⁺ channel domain predicted the presence of six (78) or eight (33) transmembrane-spanning segments that are presumed to be assembled in the ER membrane to constitute the Ca²⁺ channel. A recent detailed study (79) on the transmembrane topology of the mouse type 1 IP₃ receptor confirmed the six transmembrane-spanning segments model: the six transmembrane segments (M1 to M6) and a putative pore-forming region between M5 and M6 segments constitute the Ca²⁺ channel. Furthermore, two N-glycosylation sites were located in the luminal loop between the M5 segment and the pore-forming region. The two corresponding glycosylation sites exist in rat (25) and human (26) type 1 IP₃ receptors and two consensus sites for N-glycosylation are found in rat (27) and human (28) type 2 IP₃ receptors at the positions equivalent to those in type 1 IP₃ receptor. Only one corresponding site is found in rat (29) and human (28) type 3 IP₃ receptors. The transmembrane-spanning segments are also supposed to be required for the oligomerization of four IP₃ receptor subunits (70). The function of the cytoplasmic COOH-terminal tail is

unclear. Its involvement in the Ca²⁺ channel activity is, however, suggested since a monoclonal antibody recognizing a portion of the COOH-terminus was reported to block generation of Ca²⁺ wave and oscillation in fertilized hamster eggs (80). Thus, the IP₃ receptor-Ca²⁺ channel complex is composed of four subunits, each containing six transmembrane-spanning segments, and belongs to the superfamily of voltage-gated (81) and second messenger-gated channels (82).

IV. Function of IP₃ receptor

1. Ca²⁺ channel

As evidenced by the ligand binding data, the purified IP₃ receptor clearly satisfies the properties of a physiological receptor for IP₃. That both IP₃ receptor and Ca²⁺ release channel reside in the same protein was first suggested by single channel analyses using planer lipid bilayers reconstituted with microsomal proteins from aortic smooth muscle (74). The experiments with purified type 1 IP₃ receptor incorporated into reconstituted vesicles (83) or planer lipid bilayers (66, 84) further provided evidence that both IP₃ receptor and Ca²⁺ channel are integrated in the same molecule. Thus, the IP₃ receptor is analogous to other ligand-gated ion channels except for its primary association with intracellular membranes.

Our current knowledge about the Ca²⁺ channel properties of IP₃ receptor has been provided mainly from single channel analyses using planer lipid bilayers reconstituted with crude microsomal proteins from cerebellum (for a review, see ref. 8). The channel properties of type 2 and type 3 IP₃ receptors are largely unknown. The Ca²⁺ channel of the cerebellar IP₃ receptor (type 1) is activated by IP₃, the only known physiological activator, with an EC₅₀ of around 0.2 μM: it can maximally activate the channel at 1 μM. The IP₃ receptor, when activated, can conduct all four alkaline earth cations with conductances in the order of Ba²⁺ > Sr²⁺ > Ca²⁺ > Mg²⁺. The ryanodine receptor can also conduct these divalent cations in this order, although conductances are approximately twice as large for the ryanodine receptor. Both channels also have a similar selectivity for divalent cations over monovalent cations, but their selectivities are much lower than the extreme selectivity found with plasma membrane L-type Ca²⁺ channels, implying different mechanisms of ion permeation between the two intracellular and the plasma membrane Ca²⁺ channels. Thus, the Ca²⁺ channel of the IP₃ receptor as well as the ryanodine receptor is a rather nonspecific cation selective channel permeable to Ca²⁺ and monovalent cations, quite a contrast to the actually absolute Ca²⁺-selective nature of the L-type Ca²⁺ channel.

The observed similarity of Ca²⁺ channel properties of

the IP₃ receptor and the ryanodine receptor is not surprising because these two channels exhibit a remarkable homology in the transmembrane-spanning regions as noted above (44). However, under a physiological condition in the presence of symmetrical 110 mM K⁺ and luminal free Ca²⁺ concentration of 2.5 mM, unitary Ca²⁺ current through the IP₃ receptor was estimated to be about 0.5 pA (85), an estimate fourfold lower than that through the ryanodine receptor under the identical conditions. Furthermore, the mean open time of the IP₃ receptor under physiological conditions is 3.7 msec (85), while that of the ryanodine receptor is about 20 msec, 5 times longer than that of the IP₃ receptor. Thus, the two intracellular Ca²⁺ channels differ in the amount of Ca²⁺ released upon opening: the ryanodine receptor discharges about 20 times more Ca²⁺ per each opening than the IP₃ receptor through which 5,400 Ca²⁺ ions are estimated to be released at every channel opening (85).

2. Modulation by cytoplasmic Ca²⁺

Cytoplasmic Ca²⁺ was once shown to inhibit IP₃-induced Ca²⁺ release in cerebellar microsomes with an IC₅₀ of 300 nM by decreasing IP₃ binding to microsomes (18). However, experiments with permeabilized smooth muscle revealed a biphasic effect of cytoplasmic Ca²⁺ on the IP₃-induced Ca²⁺ release with a maximum rate of release occurring at 300 nM Ca²⁺ (86). Experiments with caged Ca²⁺ that permitted analysis with a much improved time resolution indicated that both activating and inhibitory effects of Ca²⁺ on the IP₃-induced Ca²⁺ release in permeabilized smooth muscle were extremely rapid, which would allow for immediate feedback control of IP₃-induced Ca²⁺ release (87).

The regulatory effects of Ca²⁺, either activation or inhibition, seems to be produced by direct action of Ca²⁺ on the receptor since it is very fast and reversible, and it also occurs in a reconstituted system using planar lipid bilayers (88). The observations that the purified IP₃ receptor binds Ca²⁺ (89), and at least one Ca²⁺ binding site resides in the coupling domain of the receptor (90), may lend support to the idea of a direct effect of Ca²⁺. Although the existence of a Ca²⁺-binding protein, designated as calmedin, was proposed to explain the inhibitory action of Ca²⁺ on IP₃ binding to the cerebellar microsomes (91), its identification and physiological significance remain to be elucidated. Recent experiments with the purified IP₃ receptor from *Xenopus* oocytes suggested that some associated protein or factor mediates the inhibitory action of Ca²⁺ but the activating site is located on the IP₃ receptor molecule (91).

The bell-shaped dependence on cytoplasmic Ca²⁺ is also shared by the ryanodine receptor (88). However, the inhibitory action of Ca²⁺ on the receptor is exerted only

in the millimolar range, much higher than the physiological concentrations. The Ca^{2+} -release from the ryanodine receptor, therefore, displays only Ca^{2+} -dependent activation in the physiological range of cytoplasmic Ca^{2+} concentrations, while in contrast, the IP_3 receptor exhibits both Ca^{2+} -dependent activation and inhibition in the physiological concentrations of Ca^{2+} . The Ca^{2+} -dependent activation of IP_3 receptors also suggests that the IP_3 receptor can act as a Ca^{2+} -induced Ca^{2+} release channel in the presence of a fixed concentration of IP_3 if the cytoplasmic Ca^{2+} concentration is less than 300 nM. The property of IP_3 receptor as a Ca^{2+} -induced Ca^{2+} release channel is well-documented in experiments with single smooth muscle cells: a slow rise in intracellular Ca^{2+} in agonist-stimulated cells was followed by a wave of rapid regenerative Ca^{2+} release as the local Ca^{2+} concentration reached a critical concentration of approximately 160 nM (92). Recent developments of Ca^{2+} -imaging techniques revealed that many of the cells responding to IP_3 -generating external stimuli display a repetitive pattern of Ca^{2+} spikes (calcium oscillation) or Ca^{2+} oscillation propagation without decay of amplitude through the cytoplasm of the cell (calcium wave). The bell-shaped Ca^{2+} -dependence of IP_3 receptor channel opening is now generally recognized as an essential mechanism that is required for generation of the calcium wave and oscillation (for reviews, see refs. 2–4).

3. Modulation by adenine nucleotide

ATP was initially found as a cofactor necessary for IP_3 -induced Ca^{2+} release (93). The action of ATP on IP_3 receptor was studied in detail by experiments using IP_3 receptor purified from the cerebellum and incorporated into liposomes (94). Supporting the earlier observations, 10 μM ATP or nonhydrolyzable ATP analogs markedly potentiated IP_3 -induced Ca^{2+} flux into the reconstituted lipid vesicles. AMP and GTP were without effect in the activation of the IP_3 receptor. The submillimolar concentrations of ATP and its nonhydrolyzable analogs also potentiated the IP_3 -induced Ca^{2+} release in permeabilized vascular smooth muscle cells (95). Single channel data analysis indicated that ATP activated the IP_3 receptor by increasing both open probability and open time (96).

A specific binding site for ATP was detected in purified type 1 IP_3 receptor subunit (65), and two consensus sequences for ATP binding site were found in its amino acid sequence (24, 25), both located in the coupling domain of the receptor. The other two subtypes of IP_3 receptor, type 2 (27, 28) and type 3 (28–30), also contain a consensus sequence for ATP binding site in positions corresponding to that in type 1 IP_3 receptor, implying that these two subtypes are also under regulation by ATP.

Since the binding of ATP to IP_3 receptor did not affect IP_3 binding, it is reasonable to speculate that ATP binding to the coupling domain of IP_3 receptor affects in an allosteric manner the coupling efficacy of IP_3 binding and Ca^{2+} channel opening.

Interestingly, the activity of the ryanodine receptor is also known to be allosterically potentiated by submillimolar levels of ATP (97): ATP potentiates the channel by increasing both open time and open probability. This form of regulation by ATP suggests a close coupling between intracellular Ca^{2+} release and cellular energy level, and it may play a role in the preservation of cells under energy starvation such as those in ischemic heart and brain by reducing intracellular Ca^{2+} release, leading to reduced consumption of ATP and prevention of Ca^{2+} overload.

4. Modulation by phosphorylation

Earlier reports have already shown that cerebellar P_{400} protein (98) or PCPP-260 protein (99), both now known to be identical with type 1 IP_3 receptor, is one of the good substrates for PKA. Detailed biochemical analyses performed after purified type 1 IP_3 receptor became available revealed that it was also a substrate for PKC and CaMKII in addition to being a substrate for PKA (100). Phosphorylation of the receptor by these kinases was stoichiometric (one phosphate/one receptor subunit) and additive, indicating the presence of distinct phosphorylation sites for different kinases (100). Two putative phosphorylation sites for PKA were also found in the amino acid sequence of the type 1 IP_3 receptor, both being located in the coupling domain (24, 25) (Fig. 1). One of the two sites (Ser-1755, numbering based on that of rat type 1 IP_3 receptor (25)) was found to be preferentially phosphorylated by PKA, while another site (Ser-1589) became phosphorylated when the concentration of PKA was increased (100, 101). Splicing of the S2 segment (coding for 40 amino acids) located between the two phosphorylation sites for PKA seems to affect phosphorylation by PKA: type 1 IP_3 receptor purified from cerebellum, i.e., S2⁺ isoform, was phosphorylated primarily on Ser-1755, whereas the receptor purified from vas deferens, i.e., S2[−] isoform, was phosphorylated almost exclusively on Ser-1589 (36). The different tissue distribution of S2 splicing variants and their differential phosphorylation by PKA may contribute to tissue-specific regulation of IP_3 receptor activity by cAMP signaling pathways.

The phosphorylation of type 1 IP_3 receptor by PKG was originally demonstrated in vascular smooth muscle (102). A 240-kDa protein, found as a PKG substrate whose phosphorylation was in a close association with the stimulation of plasma membrane Ca^{2+} pump by the kinase (102), was later shown to be identical with type 1

IP₃ receptor (22, 42). The phosphorylation of the type 1 IP₃ receptor by PKG was independently demonstrated by Komalavilas and Lincoln (103). The receptor purified from cerebellum (S2⁻ isoform) was phosphorylated by PKG almost exclusively on Ser-1755, corresponding to one of the two phosphorylation sites for PKA. Phosphorylation of the IP₃ receptor in intact vascular smooth muscle in response to cGMP-generating vasodilators or metabolically resistant cGMP analogs has been recently demonstrated (104). The IP₃ receptor was similarly phosphorylated by stimulation with forskolin, an agent that directly activates adenylate cyclase and increases cAMP level. However, the sensitivities of these phosphorylations to various protein kinase inhibitors suggest that PKG mediates phosphorylation of IP₃ receptor in response to increases of both cAMP and cGMP in intact cells (104). Thus, in some cell types such as the vascular smooth muscle cell, it is conceivable that both cAMP and cGMP signaling pathways regulate IP₃ receptor activity through activation of PKG and the resultant phosphorylation of the receptor.

The functional consequences of phosphorylation of IP₃ receptor are equivocal. The phosphorylation of IP₃ receptor in microsomes from cerebellum resulted in a decreased sensitivity of IP₃-induced Ca²⁺ release to IP₃ without any effect of IP₃ binding to the receptor (105). Similar inhibition provoked by PKA phosphorylation of IP₃ receptor was also demonstrated in platelet membranes (106). Both cAMP and cGMP or agents that increase one or both cyclic nucleotides were shown to inhibit IP₃-induced Ca²⁺ release in intact or permeabilized gastric smooth muscle (107). In contrast to these observations, activation of either PKA or PKG has been shown to cause potentiation of IP₃-induced Ca²⁺ release in permeabilized hepatocytes (108–110). A recent study with affinity purified homotetrameric type 1 IP₃ receptor incorporated into reconstituted lipid vesicles indicated that the rate and extent of Ca²⁺ influx into the vesicles increased 20% after phosphorylation of the receptor by the catalytic subunit of PKA (111). Thus, phosphorylation of IP₃ receptor by PKG or PKA appears to cause changes of opposite directions depending on tissues and preparations. It should be noted that type 2 and type 3 IP₃ receptors do not have consensus sequences for phosphorylation by PKA (27–30). Accordingly, these subtypes are not expected to be phosphorylated by PKA, but their possible association with type 1 IP₃ receptor subtype in a heterotetrameric IP₃ receptor channel would further complicate the changes elicited by the phosphorylation of IP₃ receptor. Elucidation of physiological significance of the role of phosphorylation of IP₃ receptor obviously requires further studies.

Phosphorylation of IP₃ receptor by PKC and CaMKII

may provide additional pathways of feedback regulation of IP₃ receptor activity since the two protein kinases are presumed to play important roles in the IP₃ signaling pathway. The finding that the type 1 IP₃ receptor possesses protein kinase activity and autophosphorylates itself (112) further complicates the regulation of IP₃ receptor by phosphorylation. The physiological significance of the autophosphorylation is unclear. The possible role of tyrosine kinase in the regulation of IP₃ receptor activity has also been suggested (113): T cell receptor stimulation triggered a physical association of the non-receptor tyrosine kinase *Fyn* and the IP₃ receptor, resulting in tyrosine phosphorylation and activation of IP₃ receptor.

5. Modulation by protein-protein interaction

Type 1 IP₃ receptor purified from mouse cerebellum was demonstrated to bind to CaM (66) and a putative amino acid sequence responsible for binding to CaM was determined (114). However, the calmodulin binding property was not demonstrated in the purified IP₃ receptor from either rat or porcine cerebellum (18, 22, 42). Islam et al. (42) demonstrated that the CaM-binding is a property intrinsic to the type 1 IP₃ receptor expressed in non-neuronal tissues which lacks the S2 splicing segment. A sequence that characterizes the CaM binding domains of representative CaM-binding proteins such as plasma membrane Ca²⁺-pump ATPase and myosin light chain kinase is found in the type 1 IP₃ receptor of porcine aorta, and it is postulated to represent the calmodulin binding site of the receptor. In the cerebellar IP₃ receptor that contains the S2 splicing segment, the sequence is separated at the mid-point through insertion of the S2 splicing segment. This may be the reason why the CaM-binding property is lacking in the cerebellar type 1 IP₃ receptor. The physiological significance of CaM binding to the IP₃ receptor remains to be clarified.

The ryanodine receptor also contains calmodulin binding domains (115). The postulate that Ca²⁺ release through the receptor is negatively regulated by CaM (116) has been recently demonstrated in the photoresponse process in *Drosophila* photoreceptors (117).

It has been recently reported that the immunophilin protein FKBP12 is physically associated with the IP₃ receptor and the ryanodine receptor (118). The binding of an immunosuppressant drug, FK506, to the FKBP12 protein resulted in disruption of the complex. The dissociation of FKBP12 protein from the receptor led to potentiation of Ca²⁺ channel activity in either receptor. Furthermore, calcineurin, a Ca²⁺-dependent protein phosphatase, was shown to be associated with the IP₃ receptor- and the ryanodine receptor-FKBP12 complexes (119). Thus, calcineurin which is anchored to the IP₃ receptor or the ryanodine receptor via FKBP12 protein

may regulate the phosphorylation state of the receptors.

V. Conclusion

The existence of multiple IP₃ receptor subtypes with different cellular and subcellular distributions, along with the remarkable complexities in regulation mechanisms inherent to the respective receptor subtypes, predict the differential regulation of Ca²⁺ signaling in distinct cells in response to external stimuli which act through the production of IP₃. The extensive studies conducted over the past decade, with the aid of the biochemical, physiological, pharmacological techniques or their combination, have provided basic knowledge about fundamental aspects of the structure and function of the IP₃ receptor. In particular the biphasic regulation of IP₃ receptor by cytoplasmic Ca²⁺ is now generally recognized as an essential mechanism for the generation of calcium waves and oscillations. However, questions about the functional differences between the various IP₃ receptor subtypes and the physiological significance of a great number of regulatory mechanisms postulated for regulation of IP₃ receptor activity largely remain to be answered.

REFERENCES

- Carafoli E: Intracellular calcium homeostasis. *Annu Rev Biochem* **56**, 395–433 (1987)
- Berridge MJ: Inositol trisphosphate and calcium signalling. *Nature* **361**, 315–325 (1993)
- Rooney TA and Thomas AP: Intracellular calcium waves generated by Ins(1,4,5)-dependent mechanisms. *Cell Calcium* **14**, 674–690 (1993)
- Clapham DE: Calcium signaling. *Cell* **80**, 259–268 (1995)
- Ferris CD and Snyder SH: Inositol 1,4,5-trisphosphate-activated calcium channels. *Annu Rev Physiol* **54**, 469–488 (1992)
- Mikoshiba K: Inositol 1,4,5-trisphosphate receptor. *Trends Pharmacol Sci* **14**, 86–88 (1993)
- Furuichi T and Mikoshiba K: Inositol 1,4,5-trisphosphate receptor-mediated Ca²⁺ signaling in the brain. *J Neurochem* **64**, 953–960 (1995)
- Bezprozvanny I and Ehrlich BE: The inositol 1,4,5-trisphosphate (InsP₃) receptor. *J Membr Biol* **145**, 205–216 (1995)
- Berridge MJ: Inositol trisphosphate and calcium signaling. *Ann NY Acad Sci* **766**, 31–43 (1995)
- Joseph SK: The inositol triphosphate receptor. *Cell Signal* **8**, 1–7 (1996)
- Sternweis PC and Smrcka AV: Regulation of phospholipase C by G proteins. *Trends Biochem Sci* **17**, 502–506 (1992)
- Neer EJ: G proteins: organizers of transmembrane signals. *Cell* **80**, 249–257 (1995)
- Moriarty TM, Padrell E, Carty DJ, Omri G, Landau EM and Lyengar R: G_o protein as signal transducer in the pertussis toxin-sensitive phosphatidylinositol pathway. *Nature* **343**, 79–82 (1990)
- Pawson T: Protein modules and signalling network. *Nature* **373**, 573–580 (1995)
- Renard DC, Bolton MM, Rhee SG, Margolis BL, Zilberstein A, Schlessinger J and Thomas AP: Modified kinetics of platelet-derived growth factor-induced Ca²⁺ increases in NIH-3T3 cells overexpressing phospholipase C γ 1. *Biochem J* **281**, 775–784 (1992)
- Kato H, Fukami K, Shibasaki K, Homma Y and Takenawa T: Enhancement of phospholipase C δ 1 activity in the aortas of spontaneously hypertensive rats. *J Biol Chem* **267**, 6483–6487 (1992)
- Kanematsu T, Takeya H, Watanabe Y, Ozaki S, Yoshida M, Koga T, Iwanaga S and Hirata M: Putative inositol 1,4,5-trisphosphate binding proteins in rat brain cytosol. *J Biol Chem* **267**, 6518–6525 (1992)
- Supattapone S, Worley PF, Baraban JM and Snyder SH: Solubilization, purification, and characterization of an inositol trisphosphate receptor. *J Biol Chem* **263**, 1530–1534 (1988)
- Maeda N, Niinobe M and Mikoshiba K: A cerebellar Purkinje cell marker P₄₀₀ protein is an inositol 1,4,5-trisphosphate (InsP₃) receptor protein. Purification and characterization of InsP₃ receptor complex. *EMBO J* **9**, 61–67 (1990)
- Chadwick CC, Saito A and Fleischer S: Isolation and characterization of the inositol trisphosphate receptor from smooth muscle. *Proc Natl Acad Sci USA* **87**, 8747–8750 (1990)
- Mourey R, Verma A, Supattapone S and Snyder SH: Purification and characterization of the inositol 1,4,5-trisphosphate receptor protein from rat vas deferens. *Biochem J* **272**, 383–389 (1990)
- Koga T, Yoshida Y, Cai JQ, Islam MO and Imai S: Purification and characterization of 240-kDa cGMP-dependent protein kinase substrate of vascular smooth muscle: Close resemblance to inositol 1,4,5-trisphosphate receptor. *J Biol Chem* **269**, 11640–11647 (1994)
- O'Rourke F, Matthews E and Feinstein MB: Purification and characterization of the human type 1 Ins(1,4,5)P₃ receptor from platelets and comparison with receptor subtypes in other normal transformed blood cells. *Biochem J* **312**, 499–503 (1995)
- Furuichi T, Yoshikawa S, Miyawaki A, Wada K, Maeda N and Mikoshiba K: Primary structure and functional expression of the inositol 1,4,5-trisphosphate-binding protein P₄₀₀. *Nature* **342**, 32–38 (1989)
- Mignery GA, Newton CL, Archer BT III and Südhof TC: Structure and expression of the rat inositol 1,4,5-trisphosphate receptor. *J Biol Chem* **265**, 12679–12685 (1990)
- Yamada N, Makino Y, Clark RA, Pearson DW, Mattei MG, Guénet JL, Ohama E, Fujino I, Miyawaki A, Furuichi T and Mikoshiba K: Human inositol 1,4,5-trisphosphate type 1 receptor, InsR1: structure, function, regulation of expression and chromosomal localization. *Biochem J* **302**, 781–790 (1994)
- Südhof TC, Newton CL, Archer BT III, Ushkarov YA and Mignery GA: Structure of a novel InsP₃ receptor. *EMBO J* **10**, 3199–3206 (1991)
- Yamamoto-Hino M, Sugiyama T, Hikichi K, Mattei MG, Hasegawa K, Sekine S, Sakurada K, Miyawaki A, Furuichi T, Hasegawa M and Mikoshiba K: Cloning and characterization of human type 2 and type 3 inositol 1,4,5-trisphosphate receptors. *Recept Channel* **2**, 9–22 (1994)
- Blondel O, Takeda J, Janssen H, Seino S and Bell GI: Sequence and functional characterization of a third inositol trisphosphate receptor subtype, IP₃R-3, expressed in pancreatic islets, kidney, gastrointestinal tract, and other tissues. *J Biol Chem* **268**,

- 11356–11363 (1993)
- 30 Maranto AT: Primary structure, ligand binding, and localization of the human type 3 inositol 1,4,5-trisphosphate receptor expressed in intestinal epithelium. *J Biol Chem* **269**, 1222–1230 (1994)
 - 31 Ross CA, Danoff SK, Schell MJ, Snyder SH and Ullrich A: Three additional inositol 1,4,5-trisphosphate receptors: molecular cloning and differential localization in brain and peripheral tissues. *Proc Natl Acad Sci USA* **89**, 4265–4269 (1992)
 - 32 De Smedt H, Missiaen L, Parys JB, Bootman MD, Mertens L, Van Den Bosch L and Casteels R: Determination of relative amounts of inositol trisphosphate receptor mRNA isoforms by ratio polymerase chain reaction. *J Biol Chem* **269**, 21691–21698 (1994)
 - 33 Mignery GA and Südhof TC: The ligand binding site and transduction mechanism in the inositol 1,4,5-trisphosphate receptor. *EMBO J* **9**, 3893–3898 (1990)
 - 34 Furuichi T, Kohda K, Miyawaki A and Mikoshiba K: Intracellular channels. *Curr Opin Neurobiol* **4**, 294–303 (1994)
 - 35 Nakagawa T, Okano H, Furuichi T, Aruga J and Mikoshiba K: The subtypes of the inositol 1,4,5-trisphosphate receptor are expressed in a tissue-specific and developmentally specific manner. *Proc Natl Acad Sci USA* **88**, 6244–6248 (1991)
 - 36 Danoff SK, Ferris CD, Donath C, Fischer CA, Munemitsu S, Ullrich A, Snyder SH and Ross CA: Inositol 1,4,5-trisphosphate receptors: Distinct neuronal and nonneuronal forms derived by alternative splicing differ in phosphorylation. *Proc Natl Acad Sci USA* **88**, 2951–2955 (1991)
 - 37 Schell MJ, Danoff SK, and Ross CA: Inositol (1,4,5)-trisphosphate receptor: characterization of neuron-specific alternative splicing in rat brain and peripheral tissues. *Mol Brain Res* **17**, 212–216 (1993)
 - 38 Newton CL, Mignery GA and Südhof TC: Co-expression of vertebrate tissues and cell lines of multiple inositol 1,4,5-trisphosphate (InsP₃) receptors with distinct affinities for InsP₃. *J Biol Chem* **269**, 28613–28619 (1994)
 - 39 Furuichi T, Simon-Chazottes D, Fujino I, Yamada N, Hasegawa M, Miyawaki A, Yoshikawa S, Guénet JL and Mikoshiba K: Widespread expression of inositol 1,4,5-trisphosphate receptor type 1 gene (*Insp3r1*) in the mouse central nervous system. *Recept Channels* **1**, 11–24 (1993)
 - 40 Wojcikiewicz RJH: Type I, II and III inositol 1,4,5-trisphosphate receptors are unequally susceptible to down-regulation and are expressed in markedly different proportions in different cell types. *J Biol Chem* **270**, 11678–11683 (1995)
 - 41 Nakagawa T, Shiota C, Okano H and Mikoshiba K: Differential localization of alternative spliced transcripts encoding inositol 1,4,5-trisphosphate receptors in mouse cerebellum and hippocampus: in situ hybridization study. *J Neurochem* **57**, 1807–1810 (1991)
 - 42 Islam MO, Yoshida Y, Koga T, Kojima M, Kangawa K and Imai S: Isolation and characterization of vascular smooth muscle inositol 1,4,5-trisphosphate receptor. *Biochem J* **316**, 295–302 (1996)
 - 43 Yamamoto-Hino M, Miyawaki A, Kawano H, Sugiyama T, Furuichi T, Hasegawa M and Mikoshiba K: Immunohistochemical study of inositol 1,4,5-trisphosphate receptor type 3 in rat central nervous system. *Neuroreport* **6**, 273–276 (1995)
 - 44 Mignery GA, Südhof TC, Takei K and De Camilli P: Putative receptor for inositol 1,4,5-trisphosphate similar to ryanodine receptor. *Nature* **342**, 192–195 (1989)
 - 45 Otsu H, Yamamoto A, Maeda N, Mikoshiba K and Tashiro Y: Immunogold localization of inositol 1,4,5-trisphosphate (InsP₃) receptor in mouse cerebellar Purkinje cells using three antibodies. *Cell Struct Funct* **15**, 163–173 (1990)
 - 46 Satoh T, Ross CA, Villa A, Supattapone S, Pozzan T, Snyder SH and Meldolesi J: The inositol 1,4,5-trisphosphate receptor in cerebellar Purkinje cells. Quantitative immunogold labeling reveals concentration in an endoplasmic reticulum subcompartment. *J Cell Biol* **111**, 615–624 (1990)
 - 47 Rusakov DA, Podini P, Villa A and Meldolesi J: Tridimensional organization of Purkinje neuron cisternal stacks, a specialized endoplasmic reticulum subcompartment rich in inositol 1,4,5-trisphosphate receptors. *J Neurocytol* **22**, 273–282 (1993)
 - 48 Fujimoto T, Nakade S, Miyawaki A, Mikoshiba K and Ogawa K: Localization of inositol 1,4,5-trisphosphate receptor-like protein in plasmalemmal caveolae. *J Cell Biol* **119**, 1507–1513 (1992)
 - 49 Villa A, Podini P, Panzeri MC, Soling HD, Volpe P and Meldolesi J: The endoplasmic-sarcoplasmic reticulum of smooth muscle: immunocytochemistry of vas deferens fibers reveals specialized subcompartments differently equipped for the control of Ca²⁺ homeostasis. *J Cell Biol* **121**, 1041–1051 (1993)
 - 50 Nakanishi S, Fujii A, Nakade S and Mikoshiba K: Immunohistochemical localization of inositol 1,4,5-trisphosphate receptors in non-neural tissues, with special reference to epithelia, the reproductive system, and muscular tissues. *Cell Tissue Res* **285**, 235–251 (1996)
 - 51 Kijima Y, Saito A, Jetton TL, Magnuson MA and Fleischer S: Differential intracellular localization of inositol 1,4,5-trisphosphate and ryanodine receptors in cardiomyocytes. *J Biol Chem* **268**, 3499–3506 (1993)
 - 52 Guillemette G, Balla T, Baukal AJ and Catt KJ: Characterization of inositol 1,4,5-trisphosphate receptors and calcium mobilization in a hepatic plasma membrane fraction. *J Biol Chem* **263**, 4541–4548 (1988)
 - 53 Rossier MF, Bird GS and Putney JW Jr: Subcellular localization of the calcium-storing inositol 1,4,5-trisphosphate-sensitive organelle in rat liver. Possible linkage to the plasma membrane through the actin microfilaments. *Biochem J* **274**, 643–650 (1991)
 - 54 Sharp AH, Snyder SH and Nigam SK: Inositol 1,4,5-trisphosphate receptors. Localization in epithelial tissues. *J Biol Chem* **267**, 7444–7449 (1992)
 - 55 Livremont JP, Hill AM, Hilly M and Mauger JP: The inositol 1,4,5-trisphosphate receptors is localized on specialized subregions of the endoplasmic reticulum in rat liver. *Biochem J* **300**, 419–427 (1994)
 - 56 Joseph SK and Samanta S: Detergent solubility of the inositol trisphosphate receptor in rat brain membranes: evidence for association of the receptor with ankyrin. *J Biol Chem* **268**, 6477–6486 (1993)
 - 57 Bourguignon LYW, Jin H, Iida N, Brandt NR and Zhang SH: The involvement of ankyrin in the regulation of inositol 1,4,5-trisphosphate receptor-mediated internal Ca²⁺ release from Ca²⁺ storage vesicles in mouse T-lymphoma cells. *J Biol Chem* **268**, 7290–7297 (1993)

- 58 Bourguignon LYW and Jin H: Identification of the ankyrin-binding domain of the mouse T-lymphoma cell inositol 1,4,5-trisphosphate (IP₃) receptor and its role in the regulation of IP₃-mediated internal Ca²⁺ release. *J Biol Chem* **270**, 7257–7260 (1995)
- 59 Berridge MJ: Capacitative calcium entry. *Biochem J* **312**, 1–11 (1995)
- 60 Fujimoto T: Calcium pump of the plasma membrane is localized in caveolae. *J Cell Biol* **119**, 1507–1513 (1992)
- 61 Khan AA, Steiner JP, Klein MG, Schneider MF and Snyder SH: IP₃ receptor: Localization to plasma membrane of T cells and cocapping with the T cell receptor. *Science* **257**, 815–818 (1992)
- 62 Khan AA, Steiner JP and Snyder SH: Plasma membrane inositol 1,4,5-trisphosphate receptor of lymphocytes: Selective enrichment in sialic acid and unique binding specificity. *Proc Natl Acad Sci USA* **89**, 2849–2853 (1992)
- 63 Kuno M and Gardner P: Ion channels activated by inositol 1,4,5-trisphosphate in plasma membrane of human T-lymphocytes. *Nature* **326**, 301–304 (1987)
- 64 Khan AA, Soloski MJ, Sharp AH, Schilling G, Sabatini DM, Li SH, Ross CA and Snyder SH: Lymphocyte apoptosis: mediation by increased type 3 inositol 1,4,5-trisphosphate receptor. *Science* **273**, 503–507 (1996)
- 65 DeLisle S, Blondel O and Longo F: Expression of inositol 1,4,5-trisphosphate receptors changes the Ca²⁺ signal of *Xenopus* oocytes. *Am J Physiol* **270**, C1255–C1261 (1996)
- 66 Maeda N, Kawasaki T, Nakade S, Yokota N, Taguchi T, Kasai M and Mikoshiba K: Structural and functional characterization of inositol 1,4,5-trisphosphate receptor channel from mouse cerebellum. *J Biol Chem* **266**, 1109–1116 (1991)
- 67 Monkawa T, Miyawaki A, Sugiyama T, Yoneshima H, Yamamoto-Hino M, Furuichi T, Saruta T, Hasegawa M and Mikoshiba K: Heterotetrameric complex formation of inositol 1,4,5-trisphosphate receptor subunits. *J Biol Chem* **270**, 14700–14707 (1995)
- 68 Wojcikiewicz RJH and He Y: Type I, II and III inositol 1,4,5-trisphosphate receptor co-immunoprecipitation as evidence for the existence of heterotetrameric receptor complexes. *Biochem Biophys Res Commun* **213**, 334–341 (1995)
- 69 Joseph SK, Pierson S, Thomas AP and Maranto AR: Heterooligomers of type-I and type-III inositol trisphosphate receptors in WB rat liver epithelial cells. *J Biol Chem* **270**, 23310–23316 (1995)
- 70 Miyawaki A, Furuichi T, Ryou Y, Yoshikawa S, Nakagawa T, Saitoh T and Mikoshiba K: Structure-function relationships of the mouse inositol 1,4,5-trisphosphate receptor. *Proc Natl Acad Sci USA* **88**, 4911–4915 (1991)
- 71 Yoshikawa F, Morita M, Monkawa T, Michikawa T, Furuichi T and Mikoshiba K: Mutation analysis of the ligand binding site of the inositol 1,4,5-trisphosphate receptor. *J Biol Chem* **271**, 18277–18284 (1996)
- 72 Kobayashi S, Somlyo AV and Somlyo AP: Heparin inhibits the inositol 1,4,5-trisphosphate-dependent, but not the independent calcium release induced by guanine nucleotide in vascular smooth muscle. *Biochem Biophys Res Commun* **153**, 625–631 (1988)
- 73 Ghosh TK, Eis PS, Mullaney JM, Ebert CL and Gill DL: Competitive, reversible, and potent antagonism of inositol 1,4,5-trisphosphate activated calcium release by heparin. *J Biol Chem* **263**, 11075–11079 (1988)
- 74 Ehrlich BE and Watras J: Inositol 1,4,5-trisphosphate activates a channel from smooth muscle sarcoplasmic reticulum. *Nature* **336**, 583–586 (1988)
- 75 Ehrlich B, Kaftan E, Bezprozvannaya S and Bezprozvanny L: The pharmacology of intracellular Ca²⁺-release channels. *Trends Pharmacol Sci* **15**, 145–149 (1994)
- 76 Takahashi S, Tanzawa K and Takahashi S: Adenophostins, newly discovered metabolite of *Penicillium brevicompactum*, act as potent agonists of the inositol 1,4,5-trisphosphate receptor. *J Biol Chem* **269**, 369–372 (1994)
- 77 Wilcox RA, Erneux C, Primrose WU, Gigg R and Nahorski SR: 2-Hydroxyethyl- α -D-glucopyranoside-2',3',4'-trisphosphate, a novel, metabolically resistant, adenophostin A and myo-inositol-1,4,5-trisphosphate analogue, potently interacts with the myo-inositol-1,4,5-trisphosphate receptor. *Mol Pharmacol* **47**, 1204–1211 (1995)
- 78 Yoshikawa S, Tanimura T, Miyawaki A, Nakamura M, Yuzaki M, Furuichi T and Mikoshiba K: Molecular cloning and characterization of the inositol 1,4,5-trisphosphate receptor in *Drosophila melanogaster*. *J Biol Chem* **365**, 3199–3206 (1992)
- 79 Michikawa T, Hamanaka H, Otsu H, Yamamoto A, Miyawaki A, Furuichi T, Tashiro Y and Mikoshiba K: Transmembrane topology and sites for N-glycosylation of inositol 1,4,5-trisphosphate receptor. *J Biol Chem* **269**, 9184–9489 (1994)
- 80 Miyazaki S, Yuzaki M, Nakade K, Sirakawa H, Nakanishi S, Nakade S and Mikoshiba K: Block of Ca²⁺ wave and Ca²⁺ oscillation by antibody to the inositol 1,4,5-trisphosphate receptor in fertilized hamster eggs. *Science* **257**, 251–255 (1992)
- 81 Caterall WA: Structure and function of voltage-sensitive ion channels. *Science* **242**, 50–61 (1988)
- 82 Jan LY and Jan YN: Tracing the root of ion channels. *Cell* **69**, 715–718 (1992)
- 83 Ferris CD, Haganir RL, Supattapone S and Snyder SH: Purified inositol 1,4,5-trisphosphate receptor mediates calcium flux in reconstituted lipid vesicles. *Nature* **342**, 87–89 (1989)
- 84 Mayrleitner M, Chadwick CC, Timerman AP, Fleischer S and Schindler H: Purified IP₃ receptor from smooth muscle forms an IP₃ gated and heparin sensitive Ca²⁺ channel in planar bilayers. *Cell Calcium* **12**, 505–514 (1991)
- 85 Bezprozvanny I and Ehrlich BE: Inositol (1,4,5)-trisphosphate (InsP₃)-gated Ca channels from cerebellum: conduction properties for divalent cations and regulation by intraluminal calcium. *J Gen Physiol* **104**, 821–856 (1994)
- 86 Iino M: Biphasic Ca²⁺-dependence of inositol 1,4,5-trisphosphate-induced Ca²⁺ release in smooth muscle cells of the guinea-pig taenia caeci. *J Gen Physiol* **95**, 1103–1122 (1990)
- 87 Iino M and Endo M: Calcium-dependent immediate feedback control of inositol 1,4,5-trisphosphate-induced Ca²⁺ release. *Nature* **360**, 76–78 (1992)
- 88 Bezprozvanny I, Watras J and Ehrlich BE: Bell-shaped calcium-response curves of Ins(1,4,5)P₃- and calcium-gated channels from endoplasmic reticulum of cerebellum. *Nature* **351**, 751–754 (1991)
- 89 Danoff SK, Supattapone S and Snyder SH: Characterization of a membrane protein from brain mediating the inhibition of inositol 1,4,5-trisphosphate receptor binding by calcium. *Biochem J* **254**, 701–705 (1988)
- 90 Mignery GA, Johnston PA and Südhof TC: Mechanism of Ca²⁺ inhibition of inositol 1,4,5-trisphosphate (InsP₃) binding

- to the cerebellar InsP₃ receptor. *J Biol Chem* **267**, 9184–9189 (1992)
- 91 Callamaras N and Parker I: Inositol 1,4,5-trisphosphate receptors in *Xenopus laevis* oocytes: localization and modulation by Ca²⁺. *Cell Calcium* **15**, 60–72 (1994)
 - 92 Iino M, Yamazawa T, Miyashita Y, Endo M and Kasai H: Critical intracellular Ca²⁺ concentration for all-or-none Ca²⁺-spiking in single smooth muscle cells. *EMBO J* **12**, 5287–5291 (1993)
 - 93 Smith JB, Smith L and Higgins BL: Temperature and nucleotide dependence of calcium release by myo-inositol 1,4,5-trisphosphate in cultured vascular smooth muscle cells. *J Biol Chem* **260**, 14413–14416 (1985)
 - 94 Ferris CD, Haganir RL and Snyder SH: Calcium flux mediated by purified inositol 1,4,5-trisphosphate receptor in reconstituted lipid vesicles is allosterically regulated by adenine nucleotides. *Proc Natl Acad Sci USA* **87**, 2147–2151 (1990)
 - 95 Iino M: Effects of adenine nucleotides on inositol 1,4,5-trisphosphate-induced calcium release in vascular smooth muscle. *J Gen Physiol* **98**, 681–698 (1991)
 - 96 Bezprozvanny I and Ehrlich BE: ATP modulates the function of inositol 1,4,5-trisphosphate-gated channels at two sites. *Neuron* **10**, 1175–1184 (1993)
 - 97 Smith JS, Coronado R and Meissner G: Single channel measurements of the calcium release channels from skeletal muscle sarcoplasmic reticulum. Activation by Ca²⁺ and ATP and modulation by Mg²⁺. *J Gen Physiol* **88**, 573–588 (1986)
 - 98 Yamamoto H, Maeda N, Niinobe M, Miyamoto E and Mikoshiba K: Phosphorylation of P₄₀₀ protein by cyclic AMP-dependent protein kinase and Ca²⁺/calmodulin-dependent protein kinase II. *J Neurochem* **53**, 917–923 (1989)
 - 99 Walaas SI, Nairn AC and Greengard P: PCPP-260, a Purkinje cell-specific cyclic AMP-regulated membrane phosphoprotein of Mr. 260,000. *J Neurosci* **6**, 954–961 (1986)
 - 100 Ferris CD, Haganir RL, Bredt DS, Cameron AM and Snyder SH: Inositol 1,4,5-trisphosphate receptor: phosphorylation by protein kinase C and calcium-calmodulin dependent protein kinases in reconstituted lipid vesicles. *Proc Natl Acad Sci USA* **88**, 2232–2235 (1991)
 - 101 Ferris CD, Cameron AM, Bredt DS, Haganir RL and Snyder SH: Inositol 1,4,5-trisphosphate receptor is phosphorylated by cyclic AMP-dependent protein kinase at serines 1755 and 1589. *Biochem Biophys Res Commun* **175**, 192–198 (1991)
 - 102 Yoshida Y, Sun HT, Cai JQ and Imai S: Cyclic GMP-dependent protein kinase stimulates the plasma membrane Ca²⁺ pump ATPase of vascular smooth muscle via phosphorylation of a 240-kDa protein. *J Biol Chem* **266**, 19819–19825 (1991)
 - 103 Komalavilas P and Lincoln TM: Phosphorylation of the inositol 1,4,5-trisphosphate receptor by cyclic GMP-dependent protein kinase. *J Biol Chem* **269**, 8701–8704 (1994)
 - 104 Komalavilas P and Lincoln TM: Phosphorylation of the inositol 1,4,5-trisphosphate receptor. Cyclic GMP-dependent protein kinase mediates cAMP and cGMP dependent phosphorylation in the intact rat aorta. *J Biol Chem* **271**, 21933–21938 (1996)
 - 105 Supattapone S, Danoff S, Theibert A, Joseph SK, Steiner J and Snyder SH: Cyclic AMP-dependent phosphorylation of a brain inositol trisphosphate receptor decreases its release of calcium. *Proc Natl Acad Sci USA* **85**, 8747–8750 (1988)
 - 106 Quinton TM and Dean WL: Cyclic AMP-dependent phosphorylation of the inositol-1,4,5-trisphosphate receptor inhibits Ca²⁺ release from platelet membranes. *Biochem Biophys Res Commun* **184**, 893–899 (1992)
 - 107 Murthy KS, Severi C, Grider JR and Makhoul GM: Inhibition of IP₃ and IP₃-dependent Ca²⁺ mobilization by cyclic nucleotides in isolated gastric muscle cells. *Am J Physiol* **264**, G967–G974 (1993)
 - 108 Burgess GM, Bird GSJ, Obie JG and Putney JW: The mechanism for synergism between phospholipase C- and adenylate cyclase-linked hormones in liver. Cyclic AMP-dependent kinase augments inositol trisphosphate-mediated Ca²⁺ mobilization without increasing the cellular levels of inositol polyphosphates. *J Biol Chem* **266**, 4772–4781 (1991)
 - 109 Guihard G, Combettes L and Capiod T: 3':5'-Cyclic guanosine monophosphate (cGMP) potentiates the inositol 1,4,5-trisphosphate-evoked Ca²⁺ release in guinea pig hepatocytes. *Biochem J* **318**, 849–855 (1996)
 - 110 Rooney T, Joseph SK, Queen C and Thomas AP: Cyclic GMP induces oscillatory calcium signals in rat hepatocytes. *J Biol Chem* **271**, 19817–19825 (1996)
 - 111 Nakade S, Rhee SK, Hamanaka H and Mikoshiba K: Cyclic AMP-dependent phosphorylation of an immunopurified homotetrameric inositol 1,4,5-trisphosphate receptor (type 1) increases Ca²⁺ flux in reconstituted lipid vesicles. *J Biol Chem* **269**, 6735–6742 (1994)
 - 112 Ferris CD, Cameron AM, Bredt DS, Haganir RL and Snyder SH: Autophosphorylation of inositol 1,4,5-trisphosphate receptors. *J Biol Chem* **267**, 7036–7041 (1992)
 - 113 Jayaraman T, Ondrias K, Ondriasova E and Marks AR: Regulation of the inositol 1,4,5-trisphosphate receptor by tyrosine phosphorylation. *Science* **272**, 1492–1494 (1996)
 - 114 Yamada M, Miyawaki A, Saito K, Nakajima T, Yamamoto-Hino M, Ryo Y, Furuichi T and Mikoshiba K: The calmodulin-binding domain in the mouse type 1 inositol 1,4,5-trisphosphate receptor. *Biochem J* **308**, 83–88 (1995)
 - 115 Takeshima H, Nishimura S, Matsumoto T, Ishida H, Kangawa K, Minamino N, Matsuo H, Ueda M, Hanaoka M, Hirose T and Numa S: Primary structure and expression from complementary DNA of skeletal muscle ryanodine receptor. *Nature* **399**, 439–445 (1989)
 - 116 Sitsapesan R, McGarry SJ and Williams AJ: Cyclic ADP-ribose, the ryanodine receptor and Ca²⁺ release. *Trends Pharmacol Sci* **16**, 386–391 (1995)
 - 117 Arnon A, Cook B, Montell C, Selinger Z and Minke B: Calmodulin regulation of calcium stores in phototransduction of *Drosophila*. *Science* **275**, 1119–1121 (1997)
 - 118 Cameron AM, Steiner JP, Sabatini DM, Kaplin AI, Walensky LD and Snyder SH: Immunophilin FK506 binding protein associated with inositol 1,4,5-trisphosphate receptor modulates calcium flux. *Proc Natl Acad Sci USA* **92**, 1784–1788 (1995)
 - 119 Cameron AM, Steiner JP, Roskams AJ, Ali SM, Ronnett GV and Snyder SH: Calcineurin associated with the inositol 1,4,5-trisphosphate receptor-FKBP12 complex modulates Ca²⁺ flux. *Cell* **83**, 463–472 (1995)