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 accommodate 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

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

IV. Function of IP$_3$ receptor

1. Ca$^{2+}$ channel
2. Modulation by cytoplasmic Ca$^{2+}$
3. Modulation by adenine nucleotide
4. Modulation by phosphorylation
5. Modulation by protein-protein interaction

V. Conclusion

Abbreviations used are (in alphabetical order): CaM, calmodulin; CaMKII, Ca$^{2+}$/calmodulin-dependent protein kinase II; DAG, diacylglycerol; ER, endoplasmic reticulum; FKBP12, FK506-binding protein 12; IP$_3$, inositol 1,4,5-trisphosphate; PCR, polymerase chain reaction; PIP$_2$, phosphatidylinositol 4,5-bisphosphate; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PKG, cGMP-dependent protein kinase; PLC, phospholipase C.
1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). IP3 plays a dominant role as a second messenger molecule for the release of Ca\(^{2+}\) from intracellular stores, while DAG activates protein kinase C (PKC). It binds to an intracellular receptor (IP3 receptor) located on the surface of intracellular Ca\(^{2+}\) stores. The IP3 receptor is at the same time a release channel for luminal Ca\(^{2+}\) whose molecular and physiological properties closely resemble those of another intracellular, Ca\(^{2+}\)-mobilizing channel, i.e., the ryanodine receptor. Ca\(^{2+}\) 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 Ca\(^{2+}\) signals. Many lines of evidence now indicate that regenerative, intricately regulated Ca\(^{2+}\) release through these intracellular Ca\(^{2+}\) channels is mainly responsible for generation of intracellular Ca\(^{2+}\) 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 Ca\(^{2+}\) 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 Ca\(^{2+}\) from intracellular stores as well as the entry of external Ca\(^{2+}\). There are two major pathways for hydrolysis of PIP\(_2\) 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\(_{\beta}\) isozymes, while the tyrosine kinase-linked receptors stimulate PLC\(_{\gamma}\) isozymes to convert PIP\(_2\) to IP3 and DAG. Both pertussis toxin-sensitive and -insensitive heterotrimeric G proteins link the activation of receptors with the stimulation of PLC\(_{\beta}\) (11). Upon activation, the heterotrimeric G proteins dissociate into G\(_{\alpha}\) and G\(_{\beta\gamma}\) subunits, both of which stimulate different PLC\(_{\beta}\) isozymes (12). The involvement of pertussis toxin-insensitive G\(_{\alpha}\) subunit and other members of the family, G\(_{\alpha11}\), G\(_{\alpha14}\) and G\(_{\alpha16}\), in the stimulation of PLC\(_{\beta}\) is now well-established. The pertussis toxin-sensitive G proteins that mediate PLC stimulation are less well-characterized, although the involvement of G\(_{\alphai}\) and G\(_{\betai}\) subunits has been suggested (13). Stimulation of PLC\(_{\beta}\) by G\(_{\beta\gamma}\) subunits is now firmly established (12). G\(_{\alpha}\) and G\(_{\beta}\) subunits appear to interact with distinct sites of the PLC\(_{\beta}\) 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\(_{\gamma}\). 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\(_{\gamma}\). Interaction of PLC\(_{\gamma}\) with the specific phosphotyrosine residues of receptors brings about phosphorylation of the PLC\(_{\gamma}\) on at least 3 tyrosine residues and leads to stimulation of the enzyme and production of IP3 and DAG (2). A similar PLC\(_{\gamma}\) 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 Ca\(^{2+}\) signal is smaller though of longer duration than that due to the activation of G protein-linked receptors (4, 15).

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

<table>
<thead>
<tr>
<th>G protein-linked receptors</th>
<th>Tyrosine kinase-linked receptors</th>
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<tr>
<td>Via PLC(_{\beta})</td>
<td>Via PLC(_{\gamma})</td>
</tr>
<tr>
<td>Adrenergic: (\alpha_{1A}, \alpha_{1B}, \alpha_{1D})</td>
<td>5-HT: 5-HT(<em>{2A}), 5-HT(</em>{2B}), 5-HT(_{2C})</td>
</tr>
<tr>
<td>Muscarinic: M(_4), M(_3), M(_5)</td>
<td>Leukotriene: BLT, CysLT(_1)</td>
</tr>
<tr>
<td>Angiotensin: AT(_1)</td>
<td>Melatonin: ML(_2)</td>
</tr>
<tr>
<td>Bombesin: BB(_1), BB(_2)</td>
<td>Oxytocin: OT</td>
</tr>
<tr>
<td>Bradykinin: B(_1), B(_2)</td>
<td>Prostanoid: FP, TP, EP(_1), EP(_3)</td>
</tr>
<tr>
<td>Cholecystokinin: CCK(_A), CCK(_B)</td>
<td>Purinergic: P(<em>{2X}), P(</em>{2Y})</td>
</tr>
<tr>
<td>Endothelin: ET(_A), ET(_B)</td>
<td>Tachykinin: NK(_1), NK(_2), NK(_3)</td>
</tr>
<tr>
<td>Glutamate (EAA): mGlu(_1), mGlu(_2)</td>
<td>Vasopressin: V(<em>{1A}), V(</em>{1B})</td>
</tr>
<tr>
<td>Histamine: H(_1)</td>
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</table>

\(^a\)Receptor 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, 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 activity is reported to be increased in the aorta of spontaneously hypertensive rats (16). Recently, a high affinity binding of IP$_3$ to the PLC$_{Ca}^+$ isozyme was demonstrated (17). The phospholipase activity of the isozyme was strongly inhibited upon binding of IP$_3$.

### III. Structure and expression of IP$_3$ receptor

#### 1. Subtypes

To date, IP$_3$ receptor has been purified to apparent homogeneity from cerebellum (18, 19), smooth muscle (20–22) and platelet (23). The purified IP$_3$ 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 IP$_3$ receptor originated mostly from studies with the cerebellar receptor. Molecular cloning studies revealed the existence of at least three types of mammalian IP$_3$-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 IP$_3$ 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 IP$_3$ 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 NH$_2$-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 Ca$^{2+}$ channel domain in the COOH-terminal portion (33). The type 2 IP$_3$ 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 IP$_3$ 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 Ca$^{2+}$ channel domain (29). The ligand-binding domain is the most conserved region, whereas the coupling domain separating the ligand binding domain from the Ca$^{2+}$ 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 IP$_3$ receptor reveals a scattered and patchy distribution of conserved and variable regions (29, 30) as illustrated in Fig. 1. The ryanodine receptor, another intracellular Ca$^{2+}$-release channel, is almost twice as large as the IP$_3$ receptor; nevertheless, it has fragmental sequence homology with the IP$_3$ receptor. Interestingly, the fragmental homology is concentrated in the ligand-binding domain and in the Ca$^{2+}$ channel domain, implying their fundamental roles in the activity of the intracellular Ca$^{2+}$-release channel (34).

The type 1 IP$_3$ 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 NH$_2$-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 IP$_3$ 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 IP$_3$ 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 IP$_3$ 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 IP$_3$ 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 IP$_3$ 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 IP$_3$ receptor are shown to be expressed in a tissue-specific and
A. Domain structure

![Ligand binding](image)

B. Modulatory sites

![Corc region for IP3 binding](image)

C. Homology among subtypes

<table>
<thead>
<tr>
<th>Conserved</th>
<th>Variable</th>
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<td>1 2 3 4 5 6 7 8 9 10 11 12 13</td>
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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
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 a single 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-neuronal tissues due 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, whereas calsoesquen-trin, 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 immunohistochemical 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 tetramizes 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 Kᵦ 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

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Y. Yoshida & S. Imai
unclear. Its involvement in the Ca$^{2+}$ channel activity is, however, suggested since a monoclonal antibody recognizing a portion of the COOH-terminus was reported to block generation of Ca$^{2+}$ wave and oscillation in fertilized hamster eggs (80). Thus, the IP$_3$ receptor-Ca$^{2+}$ 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 IP$_3$ receptor

1. Ca$^{2+}$ channel

As evidenced by the ligand binding data, the purified IP$_3$ receptor clearly satisfies the properties of a physiological receptor for IP$_3$. That both IP$_3$ receptor and Ca$^{2+}$ 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 IP$_3$ receptor incorporated into reconstituted vesicles (83) or planer lipid bilayers (66, 84) further provided evidence that both IP$_3$ receptor and Ca$^{2+}$ channel are integrated in the same molecule. Thus, the IP$_3$ receptor is analogous to other ligand-gated ion channels except for its primary association with intracellular membranes.

Our current knowledge about the Ca$^{2+}$ channel properties of IP$_3$ 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 IP$_3$ receptors are largely unknown. The Ca$^{2+}$ channel of the cerebellar IP$_3$ receptor (type 1) is activated by IP$_3$, the only known physiological activator, with an EC$_{50}$ of around 0.2 nM: it can maximally activate the channel at 1 nM. The IP$_3$ receptor, when activated, can conduct all four alkaline earth cations with conductances in the order of Ba$^{2+}$ > Sr$^{2+}$ > Ca$^{2+}$ > Mg$^{2+}$. 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 Ca$^{2+}$ channels, implying different mechanisms of ion permeation between the two intracellular and the plasma membrane Ca$^{2+}$ channels. Thus, the Ca$^{2+}$ channel of the IP$_3$ receptor as well as the ryanodine receptor is a rather nonspecific cation selective channel permeable to Ca$^{2+}$ and monovalent cations, quite a contrast to the actually absolute Ca$^{2+}$-selective nature of the L-type Ca$^{2+}$ channel.

The observed similarity of Ca$^{2+}$ channel properties of the IP$_3$ 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 Ca$^{2+}$ concentration of 2.5 mM, unitary Ca$^{2+}$ current through the IP$_3$ 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 IP$_3$ 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 IP$_3$ receptor. Thus, the two intracellular Ca$^{2+}$ channels differ in the amount of Ca$^{2+}$ released upon opening: the ryanodine receptor discharges about 20 times more Ca$^{2+}$ per each opening than the IP$_3$ receptor through which 5,400 Ca$^{2+}$ ions are estimated to be released at every channel opening (85).

2. Modulation by cytoplasmic Ca$^{2+}$

Cytoplasmic Ca$^{2+}$ was once shown to inhibit IP$_3$-induced Ca$^{2+}$ release in cerebellar microsomes with an IC$_{50}$ of 300 nM by decreasing IP$_3$ binding to microsomes (18). However, experiments with permeablized smooth muscle revealed a biphasic effect of cytoplasmic Ca$^{2+}$ on the IP$_3$-induced Ca$^{2+}$ release with a maximum rate of release occurring at 300 nM Ca$^{2+}$ (86). Experiments with caged Ca$^{2+}$ that permitted analysis with a much improved time resolution indicated that both activating and inhibitory effects of Ca$^{2+}$ on the IP$_3$-induced Ca$^{2+}$ release in permeablized smooth muscle were extremely rapid, which would allow for immediate feedback control of IP$_3$-induced Ca$^{2+}$ release (87).

The regulatory effects of Ca$^{2+}$, either activation or inhibition, seems to be produced by direct action of Ca$^{2+}$ 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 IP$_3$ receptor binds Ca$^{2+}$ (89), and at least one Ca$^{2+}$ binding site resides in the coupling domain of the receptor (90), may lend support to the idea of a direct effect of Ca$^{2+}$. Although the existence of a Ca$^{2+}$-binding protein, designated as calmedin, was proposed to explain the inhibitory action of Ca$^{2+}$ on IP$_3$ binding to microsomes (91), its identification and physiological significance remain to be elucidated. Recent experiments with the purified IP$_3$ receptor from Xenopus oocytes suggested that some associated protein or factor mediates the inhibitory action of Ca$^{2+}$ but the activating site is located on the IP$_3$ receptor molecule (91).

The bell-shaped dependence on cytoplasmic Ca$^{2+}$ is also shared by the ryanodine receptor (88). However, the inhibitory action of Ca$^{2+}$ 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\(_{100}\) 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., S\(_2\) isoform, was phosphorylated primarily on Ser-1755, whereas the receptor purified from vas deferens, i.e., S\(_2\) 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. 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.

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