IP_3 Receptors: Toward Understanding Their Activation

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Inositol 1,4,5-trisphosphate receptors (IP_3R) and their relatives, ryanodine receptors, are the channels that most often mediate Ca^{2+} release from intracellular stores. Their regulation by Ca^{2+} allows them also to propagate cytosolic Ca^{2+} signals regeneratively. This brief review addresses the structural basis of IP_3R activation by IP_3 and Ca^{2+}. IP_3 initiates IP_3R activation by promoting Ca^{2+} binding to a stimulatory Ca^{2+}-binding site, the identity of which is unresolved. We suggest that interactions of critical phosphate groups in IP_3 with opposite sides of the clam-like IP_3-binding core cause it to close and propagate a conformational change toward the pore via the adjacent N-terminal suppressor domain. The pore, assembled from the last pair of transmembrane domains and the intervening pore loop from each of the four IP_3R subunits, forms a structure in which a luminal selectivity filter and a gate at the cytosolic end of the pore control cation fluxes through the IP_3R.

A BRIEF HISTORY OF IP_3 RECEPTORS

Sidney Ringer, in his famous correction to an earlier paper, showed that Ca^{2+} entry can evoke a physiological response by demonstrating that beating of the frog heart requires extracellular Ca^{2+} (Ringer 1883). Almost a century passed before it became clear that this Ca^{2+} entry, via voltage-gated Ca^{2+} channels, was not directly responsible for contraction, but instead provided the trigger for a much larger release of Ca^{2+} from stores within the sarcoplasmic reticulum (SR). The latter is mediated by type-2 ryanodine receptors (RyR) (Fabiato 1983; Cheng et al. 1993), which like many Ca^{2+} channels, are able both to transport Ca^{2+} through an open pore and respond to it. These observations highlight two general points. First, cells call upon two sources of Ca^{2+} to evoke increases in cytosolic Ca^{2+} concentration; second, interactions between these Ca^{2+} fluxes across the plasma membrane and the membranes of intracellular stores are important determinants of the physiological response. The same points apply to the Ca^{2+} signals evoked by receptors that stimulate phospholipase C (PLC) and, thereby, formation of inositol 1,4,5-trisphosphate (IP_3).

The biochemical sequence linking these receptors to formation of IP_3 emerged in the 1980s (Michell et al. 1989; Berridge 2005), but work in the decade before had established that many receptors regulate many different responses by increasing the cytosolic Ca^{2+} concentration (Rasmussen 1970; Berridge 1975). In his

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influential review, Bob Michell (Michell 1975), building on work showing that many of these receptors also stimulate phospholipid turnover (Hokin and Hokin 1953), had suggested a causal link between phosphoinositide hydrolysis and Ca^{2+} signals. Here, as in many studies, the emphasis was on Ca^{2+} entry, with a consensus only slowly emerging that Ca^{2+} fluxes across both the plasma membrane and the membranes of intracellular stores contribute to cytosolic Ca^{2+} signals (Rasmussen 1970; Berridge 1975; Williams 1980; Putney et al. 1981). In the years following Michell's review, decisive evidence, much of it coming from Mike Berridge's elegant studies of blowfly salivary gland, established that phosphoinositide hydrolysis is, as predicted by Michell, required for PLC-linked receptors to evoke Ca^{2+} signals (Berridge and Fain 1979). The same preparation was used to show that IP_3 is the first water-soluble product of the signaling pathway (Berridge 1983). IP_3, thus, emerged as a prime candidate for the cytosolic messenger linking events at the plasma membrane to release of Ca^{2+} from intracellular stores. Paradoxically, it was to be many years before the links between receptors that stimulate PLC and Ca^{2+} entry were resolved. These came with elaboration of the pathways linking empty Ca^{2+} stores to Ca^{2+} entry, the so-called store-operated Ca^{2+} entry pathway (Putney 1997; Park et al. 2009), and recognition that many trp channels are regulated by products of PLC activity (Nilius et al. 2007). IP_3 receptors (IP_3R) also contribute more directly to Ca^{2+} entry across the plasma membrane either because, at least in some cells, IP_3R are functionally expressed in the plasma membrane (Dellis et al. 2006; Dellis et al. 2008), or perhaps through their direct interactions with other plasma membrane Ca^{2+} channels (Kiselyov et al. 1999). Here, we focus solely on Ca^{2+} release from the endoplasmic reticulum (ER) by IP_3R. Some of the key steps in the evolution of our current understanding of IP_3R are listed in Table 1.

The role of the SR as the intracellular source of Ca^{2+} signals in striated muscle was long-established (Endo et al. 1970), but there was no such agreement on the identity of the organelle from which Ca^{2+} was released in other cells. Competing claims suggested roles for mitochondria or the ER. Evidence that in resting hepatocytes only the ER contains appreciable amounts of Ca^{2+} (Burgess et al. 1983) was quickly followed by the demonstration that IP_3 evoked Ca^{2+} release from a non-mitochondrial Ca^{2+} store in permeabilized pancreatic acinar cells (Streb et al. 1983). Countless groups quickly replicated these findings in many cells, and within months it was universally accepted that the ER is the major Ca^{2+} store from which IP_3 stimulates Ca^{2+} release in most animal cells (Berridge and Irvine 1984; Berridge and Irvine 1989). Subsequent work has suggested that IP_3 may also stimulate Ca^{2+} release from the Golgi apparatus (Pinton et al. 1998), from within the nucleus (Gerasimenko et al. 1995; Echevarria et al. 2003; Marchenko et al. 2005), and perhaps also from secretory vesicles (Gerasimenko et al. 1996), but ER remains the major IP_3-sensitive Ca^{2+} store. Evidence that IP_3 stimulates Ca^{2+} efflux from the ER (rather than inhibiting Ca^{2+} uptake) and the first single channel recordings (Ehrlich and Watras 1988) established that the IP_3R is an IP_3-gated, Ca^{2+}-permeable channel. The first studies of ^{32}P-IP_3 binding (Spät et al. 1986) were followed by purification of IP_3R from cerebellum (Maeda et al. 1988; Supattapone et al. 1988) and then cloning of the first IP_3R subtype (IP_3R1) (Furuichi et al. 1989; Mignery et al. 1989). Subsequent studies identified two additional genes encoding vertebrate IP_3R (IP_3R2 and IP_3R3) and a single gene in invertebrates (Taylor et al. 1999). It remains far from clear whether plants express related IP_3R (Krinke et al. 2007). These studies established that IP_3R are unusually large proteins, comprising tetramers of closely-related subunits, each with about 2700 amino acid residues. RyR are even larger: they, too, are tetramers, but the subunits are almost twice the size of IP_3R ($\sim$5000 residues). This progress with identifying IP_3R together with single channel recordings of IP_3R, initially in artificial lipid bilayers and later in native membranes (Foskett et al. 2007; Rahman et al. 2009), provided the foundations from which to explore the structural determinants of IP_3R behavior. The advances toward understanding the molecular mechanisms

Table 1. Landmarks en route to a structural analysis of IP_3 receptor behavior.

	RyR	IP_3R
1883	Ca^{2+} entry required for heart contraction.[1]	
1953		Acetylcholine stimulates turnover of phospholipids.[2]
1975		Phosphoinositide hydrolysis proposed to cause Ca^{2+} signals.[3]
1977	Ca^{2+} waves occur at fertilization.[4]	
1977	Ca^{2+}-induced Ca^{2+} release in SR.[5]	
1979		Phosphoinositide hydrolysis required for receptor-stimulated Ca^{2+} signals.[6]
1980	Introduction of Quin 2[7] and facile loading methods.[8]	
1983		IP_3 is first water-soluble product of PLC.[9]
1983		IP_3 stimulates Ca^{2+} release from a non-mitochondrial store.[10]
1985	Ryanodine, selective RyR ligand.[11]	
1985	Single channel records of RyR.[12]	
1986		Frequency-coded Ca^{2+} spikes.[13]
1987		Ca^{2+} regulates IP_3R.[14,15]
1987	RyR1 purified.[16]	IP_3R1 purified.[17]
1988		Single channel records of IP_3R.[18]
1989	Cloning of RyR1.[19]	Cloning of IP_3R1.[20,21]
1990		Elementary Ca^{2+}-release events.[22]
1993	Elementary Ca^{2+}-release events.[23]	
2002		Atomic structure of IBC.[24]
2005		Atomic structure of SD.[25]
2009	Atomic structure of N-terminal of RyR.[26,27]	

[1]Ringer (1883).
[2]Hokin & Hokin (1953).
[3]Michell (1975).
[4]Ridgeway et al. (1977).
[5]Endo (1977).
[6]Berridge & Fain (1979).
[7]Tsien (1980).
[8]Tsien (1981).
[9]Berridge (1983).
[10]Streb et al. (1983).
[11]Sutko et al. (1985).
[12]Smith et al. (1985).
[13]Woods et al. (1986).
[14]Iino (1987).
[15]Iino (1990).
[16]Imagawa et al. (1987).
[17]Supattapone et al. (1988).
[18]Ehrlich & Watras (1988).
[19]Takeshima et al. (1989).
[20]Mignery et al. (1989).
[21]Furuichi et al. (1989).
[22]Parker & Ivorra (1990).
[23]Cheng et al. (1993).
[24]Bosanac et al. (2002).
[25]Bosanac et al. (2005).
[26]Amador et al. (2009).
[27]Lobo & Van Petegem (2009).

of IP_3R behavior were accompanied by similar progress with RyR (Table 1). Recurrent themes, to which we return, are the similarities between RyR and IP_3R, and the many instances where observations of one channel family have informed further analysis of the other. Very recently, a third family of intracellular Ca^{2+} channels, unrelated to RyR and IP_3R, has been implicated in Ca^{2+} signaling. These are the two-pore channels (TPC) that are activated by NAADP and release Ca^{2+} from acidic Ca^{2+} stores, including lysosomes and endosomes (Patel et al. 2010; Zhu et al. 2010). Several trp (transient receptor protein) channels, in addition to their roles in the plasma membrane, may also mediate release of Ca^{2+} from intracellular stores (Gees et al. 2010).

Parallel to work addressing the workings of IP_3R, there was growing interest in the spatiotemporal complexity of cytosolic Ca^{2+} signals. Ca^{2+} waves were first observed during fertilization. These waves were proposed to result from Ca^{2+}-induced Ca^{2+} release (CICR) and were followed by smaller repetitive Ca^{2+} transients (Ridgway et al. 1977; Gilkey 1983). It was, however, the work of Peter Cobbold that focused most attention on the complexity of intracellular Ca^{2+} signals (Woods et al. 1986). Just as the activity of a nerve is conveyed by the frequency of its action potentials, Cobbold demonstrated that in hepatocytes the concentration of the extracellular stimulus determined the frequency of the cytosolic Ca^{2+} transients. As these ideas gathered momentum (Berridge 1995), evidence accumulated in support of cells using the information provided by frequency-encoded Ca^{2+} spikes as an efficient means of regulating cellular activity (Dolmetsch et al. 1997; Li et al. 1998; Berridge et al. 2000; Dupont et al. 2003). The single greatest contributor to progress in understanding the genesis of these intracellular Ca^{2+} signals was the introduction, by Roger Tsien in 1980, of simple, minimally disruptive methods for measuring the free cytosolic Ca^{2+} concentration in intact cells (Tsien 1980; Tsien 1981). These methods, in combination with improved optical microscopy, allowed Ian Parker to begin to resolve the subcellular organization of IP_3-evoked Ca^{2+} signals (Parker and Ivorra 1990; Parker et al. 1996). He showed that as the IP_3 concentration increases, it triggers a hierarchy of elementary Ca^{2+} release events, beginning with the openings of single IP_3R (Ca^{2+} blips), progressing to the coordinated openings of a cluster of several IP_3R (Ca^{2+} puffs) and finally, with sufficient IP_3, culminating in a regenerative Ca^{2+} wave invading the entire cell (Bootman et al. 1997; Demuro and Parker 2007). The demonstration, in 1987 by Masamitsu Iino, that IP_3R are stimulated by cytosolic Ca^{2+} (Iino 1987), and the later widespread recognition that all IP_3R are biphasically regulated by cytosolic Ca^{2+} (Iino 1990; Taylor and Laude 2002), provided what has become the most widely accepted explanation for the recruitment of elementary Ca^{2+}-release events. Namely, that CICR, already an established feature of RyR (Endo et al. 1970), allows an active IP_3R to propagate its activity to neighboring IP_3R.

These observations and accumulating evidence that local Ca^{2+} signals can selectively regulate local events (Rizzuto et al. 1993; Berridge et al. 2000; Dyer et al. 2005; Willoughby and Cooper 2007) prompted a re-assessment of the ways in which Ca^{2+} signals convey information. It became untenable to think of responses to graded changes in the intensity of the extracellular stimulus as being simply encoded in graded changes in global cytosolic Ca^{2+} concentration. Ca^{2+} entering the cytosol via one channel can regulate different proteins to Ca^{2+} entering via another (Berridge et al. 2000; Dyer et al. 2005; Willoughby and Cooper 2007). Hence, the spatial organization of the changes in cytosolic Ca^{2+} concentration profoundly affects the physiological response, and that presents many opportunities for delivering different Ca^{2+} signals in response to different stimuli or different stimulus intensities. The duration of each Ca^{2+} increase, whether local or global, is also important in determining not only the amplitude of the response, but also its nature, because Ca^{2+}-binding proteins differ in their responses to transient and sustained signals. Finally, the frequency with which Ca^{2+} signals are delivered can determine both the nature and amplitude of the cellular response. The key point is that the versatility of Ca^{2+} as an intracellular messenger

capable of regulating diverse cellular events depends largely on the spatiotemporal complexity of cytosolic Ca^{2+} signals (Berridge et al. 2000). If we are to understand how Ca^{2+} functions as a ubiquitous intracellular messenger, we must explain how IP_3-evoked Ca^{2+} signals grow from the opening of a single IP_3R to much larger events. That explanation depends, ultimately, on putting IP_3R into appropriate places within the cell, and on the interactions between IP_3 and Ca^{2+} in regulating the opening of IP_3R. In recent reviews (Taylor et al. 2009a; Taylor et al. 2009b) and original reports, we have described how IP_3R are co-translationally targeted to the ER and then retained there by sequences within their transmembrane domains (TMD) (Parker et al. 2004; Pantazaka and Taylor 2010). We have also suggested that within the ER, IP_3 causes IP_3R to assemble into small clusters within which their regulation by both IP_3 and Ca^{2+} is retuned to facilitate the Ca^{2+}-mediated recruitment of IP_3R activity by an active neighbor (Rahman and Taylor 2009; Rahman et al. 2009). Here, we focus entirely on the interactions between Ca^{2+} and IP_3 in regulating IP_3R activity, and the extent to which we can explain those interactions at the structural level.

REGULATION OF IP_3 RECEPTORS BY Ca^{2+} AND IP_3

Activation of IP_3R requires both IP_3 and its permeating ion, Ca^{2+} (Finch et al. 1991; Marchant and Taylor 1997; Adkins and Taylor 1999; Taylor and Laude 2002; Foskett et al. 2007). There are reports of IP_3-independent activation of IP_3R by CaBP1 (Yang et al. 2002), a member of the neuronal Ca^{2+}-sensor family, and by Gβγ subunits (Zeng et al. 2003), but the physiological relevance is unclear (Haynes et al. 2004; Nadif Kasri et al. 2004). The current consensus is that binding of IP_3 to the IP_3R is essential for its activation, but whether all four IP_3-binding sites of the tetrameric IP_3R must be occupied is unresolved. Positively cooperative responses to IP_3 in some (Dufour et al. 1997; Marchant and Taylor 1997; Tu et al. 2005a), though not all, studies (Finch et al. 1991; Watras et al. 1991; Laude et al. 2005), and delays before the first response to IP_3 that decrease with increasing IP_3 concentration (Marchant and Taylor 1997), indicate that channel opening requires occupancy of more than one IP_3-binding site. However, gating by IP_3 of heteromeric IP_3R in which at least one subunit is mutated to prevent IP_3 binding suggests that occupancy of fewer than four IP_3-binding sites may be sufficient to cause some channel opening (Boehning and Joseph 2000a). IP_3R subtypes differ in their affinities for IP_3, with the general consensus being that IP_3R2 is more sensitive than IP_3R1, and both are considerably more sensitive than IP_3R3 (Tu et al. 2005b; Iwai et al. 2007). In the cellular context, however, differences in expression level (Dellis et al. 2006; Tovey et al. 2010), subcellular distribution (Petersen et al. 1999), post-transcriptional and post-translational modifications, and association of IP_3R with accessory proteins (Patterson et al. 2004) may be more important determinants of sensitivity.

Soon after the first report of IP_3-evoked Ca^{2+} release, cytosolic Ca^{2+} was shown also to regulate IP_3R (Suematsu et al. 1984; Jean and Klee 1986); thereafter, it emerged that the effects of Ca^{2+} were biphasic, with modest increases in cytosolic Ca^{2+} concentration enhancing responses to IP_3, while higher concentrations were inhibitory (Iino 1987; Iino 1990; Finch et al. 1991; Parys et al. 1992; Marshall and Taylor 1993). This provided yet another parallel with RyR, which are also biphasically regulated by Ca^{2+} (Hamilton 2005). The coregulation of IP_3R by IP_3 and Ca^{2+} in permeabilized cells was confirmed by single-channel recordings of IP_3R1 reconstituted into lipid bilayers (Bezprozvanny et al. 1991; Striggow and Ehrlich 1996; Kaftan et al. 1997; Ramos-Franco et al. 1998a; Ramos-Franco et al. 1998b; Tu et al. 2002; Tu et al. 2005b) and in native nuclear membranes (Stehno-Bittel et al. 1995; Mak et al. 1998; Boehning et al. 2001a; Marchenko et al. 2005). In each case, the single-channel open probability (P_o) of IP_3-activated channels displayed a bell-shaped dependence on cytosolic Ca^{2+} concentration. Evidence that purified IP_3R1 could be stimulated, but not inhibited, by cytosolic Ca^{2+} (Thrower et al. 1998; Michikawa et al.

1999) raised the possibility that Ca^{2+} inhibition might be mediated by an accessory protein, although it has yet to be identified. The same explanation perhaps accounts for some reports, often derived from bilayer recordings, in which Ca^{2+} was suggested not to inhibit IP_3R2 or IP_3R3 (Horne and Meyer 1995; Hagar et al. 1998; Miyakawa et al. 1999; Ramos-Franco et al. 2000). The balance of opinion, supported by numerous studies of all three IP_3R subtypes and using both single-channel and Ca^{2+}-efflux studies, is that all three IP_3R subtypes are biphasically regulated by cytosolic Ca^{2+} (Marshall and Taylor 1993; Oancea and Meyer 1996; Dufour et al. 1997; Missiaen et al. 1998; Miyakawa et al. 1999; Swatton et al. 1999; Boehning and Joseph 2000b; Mak et al. 2000; Mak et al. 2001; Tu et al. 2005a). Two independent Ca^{2+}-binding sites, which differ in their interactions with different bivalent cations and in their affinities for Ca^{2+}, mediate the stimulatory and inhibitory effects of cytosolic Ca^{2+} (Marshall and Taylor 1994; Striggow and Ehrlich 1996; Hajnóczky and Thomas 1997). Both sites are essential elements of many models proposed to explain regenerative Ca^{2+} signals (Lechleiter et al. 1991; Berridge 1997). This core biphasic pattern of regulation by cytosolic Ca^{2+} may be modulated by other intracellular signals (and these, too, may have contributed to some of the disparate findings) and by processing of IP_3R. Ca^{2+}-dependent inhibition of IP_3R3, for example, is very sensitive to cytoplasmic ATP (Tu et al. 2005b), and the neuronal $S2^+$ splice variant of IP_3R1 has a broader Ca^{2+}-dependence than the peripheral $S2^-$ form (Tu et al. 2002). However, IP_3 is the major influence on what Ca^{2+} does to IP_3R: The two ligands are essential co-agonists of IP_3R (Finch et al. 1991). Activation of IP_3R1 by Ca^{2+} is positively cooperative, enabling P_o to reach its maximum value over a narrow range of Ca^{2+} concentrations, suggesting that IP_3R1 may be well suited to mediating CICR and regenerative Ca^{2+} signals. Activation of IP_3R3 is less cooperative, occurs over a broader range of Ca^{2+} concentrations, and requires lesser activation, making it well suited as a trigger for Ca^{2+} release as the level of IP_3 increases (Mak et al. 2001; Foskett et al. 2007).

Foskett and colleagues have argued, from their analyses of patch-clamp recordings of nuclear IP_3R, that IP_3 decreases the sensitivity of the IP_3R to inhibition by cytosolic Ca^{2+}, and that this alone is the means whereby IP_3 stimulates channel opening (Mak et al. 1998; Mak et al. 2001; Ionescu et al. 2006). This simple explanation, where IP_3 serves only to relieve tonic inhibition by resting Ca^{2+} concentrations, is impossible to reconcile with their observation that pretreatment of cells with Ca^{2+}-free media abolishes Ca^{2+} inhibition without preventing IP_3 from activating IP_3R (Mak et al. 2003). This simple model was later elaborated to include at least three different Ca^{2+} sensors (Mak et al. 2003), but at the core of this revised scheme is a single Ca^{2+}-binding site that switches from being inhibitory in the absence of IP_3 to stimulatory in its presence (Mak et al. 2003). The essential feature of this scheme is consistent with our initial model, derived from rapid superfusion analysis, which suggests that IP_3 both relieves Ca^{2+} inhibition and promotes binding of Ca^{2+} to a stimulatory site (Marchant and Taylor 1997; Adkins and Taylor 1999). The latter is essential for the channel to open. We, however, argue that the stimulatory and inhibitory Ca^{2+}-binding sites are distinct (Marshall and Taylor 1994). We suggest, therefore, that the essential role of IP_3 is to promote Ca^{2+} binding to a stimulatory Ca^{2+}-binding site. IP_3, by priming this site, allows Ca^{2+} to provide instantaneous control over whether the channel opens (Fig. 1A).

The structural basis for Ca^{2+}-regulation of IP_3R is unresolved: it may be either direct, via Ca^{2+} binding to a site intrinsic to the IP_3R or via an accessory Ca^{2+}-binding protein (Taylor et al. 2004). Stimulation of IP_3R by cytosolic Ca^{2+} is universally observed even with purified IP_3R reconstituted into lipid bilayers (Ferris et al. 1989; Hirota et al. 1995; Michikawa et al. 1999), suggesting that this essential Ca^{2+}-binding site probably resides within the primary sequence of the IP_3R. At least seven cytosolic Ca^{2+}-binding sites have been identified within IP_3R1 (Sienaert et al. 1996; Sienaert et al. 1997), but the physiological relevance of these sites is unresolved. Two of the sites (residues 304-381 and 378-450) are within the IP_3-binding

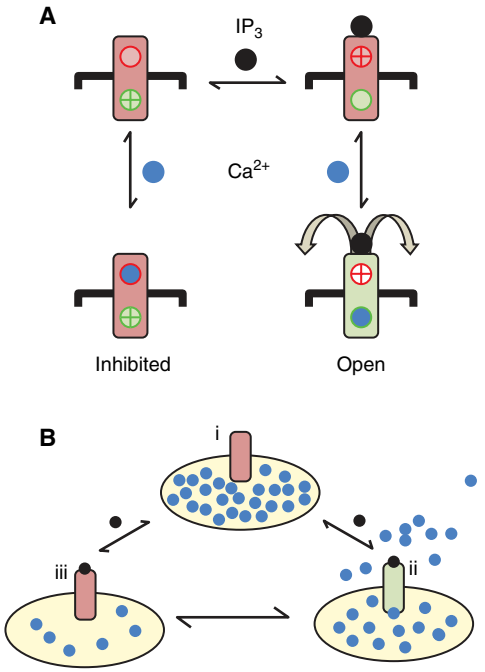

Figure 1. Regulation of IP_3R by cytosolic and luminal Ca^{2+}. (*A*) Binding of IP_3 (black circle) to the IP_3R determines whether a stimulatory (green) or inhibitory (red) Ca^{2+}-binding site is available (Adkins and Taylor 1999). IP_3 binding causes the stimulatory site to become accessible and the inhibitory site to be concealed; binding of Ca^{2+} (blue circle) to the former then triggers opening of the channel. (*B*) Luminal Ca^{2+} is proposed to tune the sensitivity of the IP_3R to cytosolic IP_3 and Ca^{2+} such that full stores (i) are most sensitive to IP_3. As the IP_3R opens (ii) and the stores lose Ca^{2+}, they are proposed to lose sensitivity to IP_3 until eventually the IP_3R closes, despite the continued presence of the cytosolic stimuli, trapping Ca^{2+} within the ER (iii). Conversely, stores regain their sensitivity to IP_3 as the stores refill, perhaps thereby determining the interval between Ca^{2+} spikes in stimulated cells (Berridge 2007).

core, for which there is a high-resolution structure (Bosanac et al. 2002). This structure shows two surface-exposed clusters of acidic residues that overlap with residues in the second N-terminal Ca^{2+}-binding region. However, point mutations of several of these acidic residues had no effect on Ca^{2+}-regulation of IP_3R (Joseph et al. 2005). The remaining Ca^{2+}-binding sites fall within the central region of the IP_3R (Sienaert et al. 1996; Sienaert et al. 1997). The site between residues 1347–1426 is interesting because its proximity to a calmodulin (CaM)-binding region is reminiscent of RyR, which have two CaM-binding regions within ~200 residues of high-affinity Ca^{2+}-binding sites, and a third flanked by two high-affinity Ca^{2+}-binding sites (Chen and MacLennan 1994). Interactions between these sites have been proposed to contribute to regulation of RyR by Ca^{2+} and CaM (Chen and MacLennan 1994). None of the Ca^{2+}-binding sites within IP_3R contain EF-hands or any other known Ca^{2+}-binding motif, and none have obvious sequence similarity with similar regions in RyR. However, each site has clusters of negatively charged residues that may coordinate Ca^{2+} (Sienaert et al. 1997). There is presently no evidence to link any of these sites directly to Ca^{2+} regulation of IP_3R. The only tangible link between specific residues and Ca^{2+} regulation comes from mutagenesis of a glutamate residue that is conserved in all IP_3R and RyR. Mutation of this residue in RyR massively reduced the Ca^{2+} sensitivity of the channel (Chen et al. 1998; Li and Chen 2001). Mutation of the same residue (Glu-2100) to another acidic residue (Asp) caused a ~5- to 10-fold decrease in the Ca^{2+}-sensitivity of the IP_3R to both stimulation and inhibition, abolished oscillatory Ca^{2+} transients in response to agonist stimulation, and reduced the Ca^{2+}-binding affinity of a large fragment that includes the residue (Miyakawa et al. 2001; Tu et al. 2003). A rather puzzling aspect of these results is the observation that mutation of a single residue similarly attenuates both stimulation and inhibition by Ca^{2+}, when other evidence suggests that the two effects are mediated by distinct sites. This, together with the lack of direct evidence that Ca^{2+} is coordinated by the conserved glutamate, leaves open the possibility that rather than itself contributing to an essential Ca^{2+}-binding site, this residue may be allosterically coupled to the site.

Ca^{2+}-mediated inhibition of IP_3R is widely assumed to contribute to termination of local cytosolic Ca^{2+} signals, but it remains far from clear whether such inhibition is mediated by Ca^{2+} binding directly to IP_3R or to an associated protein (Taylor and Laude 2002). The effects of

Ca^{2+} on IP_3 binding differ between subtypes: It inhibits binding to IP_3R1 (Worley et al. 1987; Supattapone et al. 1988; Joseph et al. 1989; Varney et al. 1990; Richardson and Taylor 1993; Benevolensky et al. 1994; Cardy et al. 1997; Yoneshima et al. 1997), but the effects of Ca^{2+} on IP_3 binding to IP_3R from cells expressing predominantly IP_3R2 or IP_3R3 are confused (Pietri et al. 1990; Mohr et al. 1993; Marshall and Taylor 1994; Cardy et al. 1997; Yoneshima et al. 1997; Lin et al. 2000; Swatton and Taylor 2002). These conflicting results, and evidence that purified IP_3R1 is not inhibited by Ca^{2+} (Danoff et al. 1988; Richardson and Taylor 1993; Benevolensky et al. 1994; Lin et al. 2000), lend some support to the idea that Ca^{2+} inhibition may be mediated by an accessory protein. It is, however, noteworthy that deletion of the suppressor domain (SD, residues 1-223) of IP_3R1, which appears not to include a Ca^{2+}-binding site, abolishes inhibition of IP_3 binding by Ca^{2+} (Sienaert et al. 2002). This suggests that effective regulation by an accessory protein might require the SD.

Calmodulin (CaM) is one candidate for the accessory protein through which Ca^{2+} inhibition is exercised (Nadif Kasri et al. 2002; Taylor and Laude 2002). CaM is a ubiquitously expressed, EF-hand containing, Ca^{2+}-binding protein that serves as the Ca^{2+}-sensor for many cellular events (Gnegy 1993). All IP_3R subtypes are inhibited by Ca^{2+}-CaM (Hirota et al. 1999; Michikawa et al. 1999; Missiaen et al. 1999; Adkins et al. 2000; Missiaen et al. 2000), and CaM has been shown to restore Ca^{2+} inhibition to purified IP_3R (Hirota et al. 1999; Michikawa et al. 1999; Nosyreva et al. 2002). Yet, it has proven difficult to relate these functional effects of CaM to either its effects on IP_3 binding or to identified CaM-binding sites within IP_3R. CaM inhibits IP_3 binding to IP_3R1 in a Ca^{2+}-independent manner (Patel et al. 1997; Cardy and Taylor 1998), through a site that probably lies within the SD (Adkins et al. 2000; Sienaert et al. 2002). Its properties are clearly inconsistent with the ability of CaM to inhibit IP_3R function only in the presence of Ca^{2+}. There is a high-affinity Ca^{2+}-CaM-binding site within the central region of IP_3R1 and IP_3R2, but not IP_3R3 (Yamada et al. 1995; Lin et al. 2000). However, mutations that prevented Ca^{2+}-CaM binding to this site had no affect on Ca^{2+}-dependent inhibition of IP_3R (Zhang and Joseph 2001; Nosyreva et al. 2002). This evidence and the absence of the site from IP_3R3 suggest that the central Ca^{2+}-CaM-binding site cannot be responsible for Ca^{2+} inhibition of IP_3R. An additional high-affinity Ca^{2+}-CaM-binding site is created in IP_3R1 after removal of the S2 splice region: While this may increase the Ca^{2+}-CaM sensitivity of peripheral $S2^-$ IP_3R1, it is not a universal candidate for mediating Ca^{2+} inhibition of IP_3R (Islam et al. 1996; Lin et al. 2000). Recently, it was suggested that bound CaM is essential for IP_3R function because a peptide antagonist of CaM inhibited IP_3-evoked Ca^{2+} release (Nadif Kasri et al. 2006). It is now clear that this peptide acts directly on IP_3R, with no requirement for CaM (Sun and Taylor 2008). While this eliminates an essential role for tethered CaM, it raises the intriguing possibility that an endogenous CaM-like structure might be essential for IP_3R activation (Sun and Taylor 2008). In summary, all IP_3R subtypes are inhibited by Ca^{2+}-CaM, but the molecular basis of this inhibition has not been established. It seems, on balance, that CaM is unlikely to be the accessory protein through which Ca^{2+} universally inhibits IP_3R. That need not preclude a role for CaM in modulating IP_3R function (Taylor and Laude 2002), just as it does for RyR (Chen et al. 1997; Fruen et al. 2000; Rodney et al. 2001), but we must look elsewhere for the site through which Ca^{2+} inhibits IP_3R.

We turn now to the luminal surface of the IP_3R, where, and again drawing parallels with RyR, we consider regulation by luminal Ca^{2+}. Persuasive evidence suggests that Ca^{2+} release by RyR may be terminated before Ca^{2+} stores are entirely depleted because luminal Ca^{2+} is required to maintain RyR activity (Györke and Györke 1998; Launikonis et al. 2006; Jiang et al. 2008), possibly via its interaction with calsequestrin, a luminal high-capacity Ca^{2+}-binding protein (Launikonis et al. 2006; Terentyev et al. 2006). A similar scheme has been proposed to account for two features of IP_3-evoked Ca^{2+} release: the initiation of Ca^{2+} release after the

quiescent interspike interval during repetitive Ca^{2+} spikes (Berridge 2007) and quantal Ca^{2+} release via IP_3R. The latter describes the situation wherein unidirectional Ca^{2+} efflux from intracellular stores terminates before the stores have fully emptied after stimulation with submaximally effective concentrations of IP_3 without loss of their ability to respond to a further increase in IP_3 concentration (Muallem et al. 1989; Meyer and Stryer 1990; Taylor and Potter 1990; Oldershaw et al. 1991; Bootman et al. 1992; Brown et al. 1992; Combettes et al. 1992; Ferris et al. 1992; Hirota et al. 1995). The proposal is that luminal Ca^{2+} sets the gain on the regulation by cytosolic IP_3 and Ca^{2+}, so that as the luminal free Ca^{2+} concentration falls, it causes the sensitivity of the IP_3R to IP_3 to fall until, as Ca^{2+} leaks from the ER, the IP_3R closes despite the continued presence of cytosolic IP_3 and residual Ca^{2+} within the ER (Irvine 1990). Conversely, as stores refill between Ca^{2+} spikes in an intact cell, the model predicts that the sensitivity of the IP_3R increases until it exceeds the threshold at which prevailing cytosolic IP_3 and Ca^{2+} concentrations become sufficient to trigger opening (Fig. 1B). Despite the enduring appeal of the model, evidence that luminal Ca^{2+} directly regulates IP_3R is not yet entirely convincing.

Stores loaded with Ca^{2+} have been shown to become more sensitive to IP_3 in some studies (Missiaen et al. 1992; Nunn and Taylor 1992; Oldershaw and Taylor 1993; Parys et al. 1993; Missiaen et al. 1994; Horne and Meyer 1995; Combettes et al. 1996; Tanimura and Turner 1996), but not in others (Combettes et al. 1992; Shuttleworth 1992; Combettes et al. 1993; van de Put et al. 1994). However, even the supportive results do not eliminate the possibility that the increased sensitivity to IP_3 arises from having Ca^{2+} pass through active IP_3R and increase their sensitivity from the cytosolic surface. Similar difficulties have plagued analyses of the effects of luminal Ca^{2+} on RyR (Tripathy and Meissner 1996; Laver 2007; Laver 2009). In bilayer recordings of IP_3R1, where essential accessory proteins may be lost, luminal Ca^{2+} failed to potentiate the Ca^{2+} release evoked by IP_3 (Bezprozvanny and Ehrlich 1994). Despite the caveats, regulation of IP_3R by luminal Ca^{2+} deserves serious consideration. A high-affinity Ca^{2+}-binding site within the luminal loop linking TMD 5 and 6 (Sienaert et al. 1996) contains conserved acidic residues that could mediate luminal Ca^{2+} regulation, although the sub-μM affinity of this site for Ca^{2+} would be ill-suited to detecting likely changes in luminal Ca^{2+} concentration. Luminal accessory proteins, akin to those that regulate RyR, are another possibility, with ERp44 being one candidate. ERp44 belongs to the thioredoxin protein family and regulates IP_3R in a pH- and luminal Ca^{2+}-dependent manner (Higo et al. 2005). Binding of ERp44 to the TMD5-6 loop of IP_3R inhibits channel activity, and the interaction is disrupted by high concentrations of Ca^{2+} consistent with the suggestion that luminal Ca^{2+} might enhance IP_3R activity.

To summarize, IP_3 works by tuning the Ca^{2+} sensitivity of the IP_3R: It stimulates Ca^{2+} binding to a stimulatory site and inhibits Ca^{2+} binding to an inhibitory site (Fig. 1A). Binding to the stimulatory site is the trigger for opening of the pore. The identity of neither Ca^{2+}-binding site is known: The stimulatory site probably resides within the IP_3R itself, but the inhibitory site may require an accessory protein, though this is unlikely to be CaM. Luminal Ca^{2+} may further tune the sensitivity of the IP_3R to regulation by its cytosolic ligands, but this remains unproven.

STRUCTURAL DETERMINANTS OF IP_3R ACTIVATION

Judged by their primary amino acid sequences, all known IP_3R subunits are assumed to have a similar architecture. Each subunit, of about 2700 residues, comprises three major regions: the N-terminal to which IP_3 binds, the C-terminal region with its six transmembrane regions (TMD) (Galvan et al. 1999), and a large intervening sequence (Fig. 2A). Functional IP_3Rs are tetrameric, assembled either from identical subunits or from mixtures of the three subtypes and their many splice variants (Taylor et al. 1999; Foskett et al. 2007). Several structures of the entire IP_3R1 have been published, each derived from single particle analysis of images from electron microscopy (Hamada and Mikoshiba

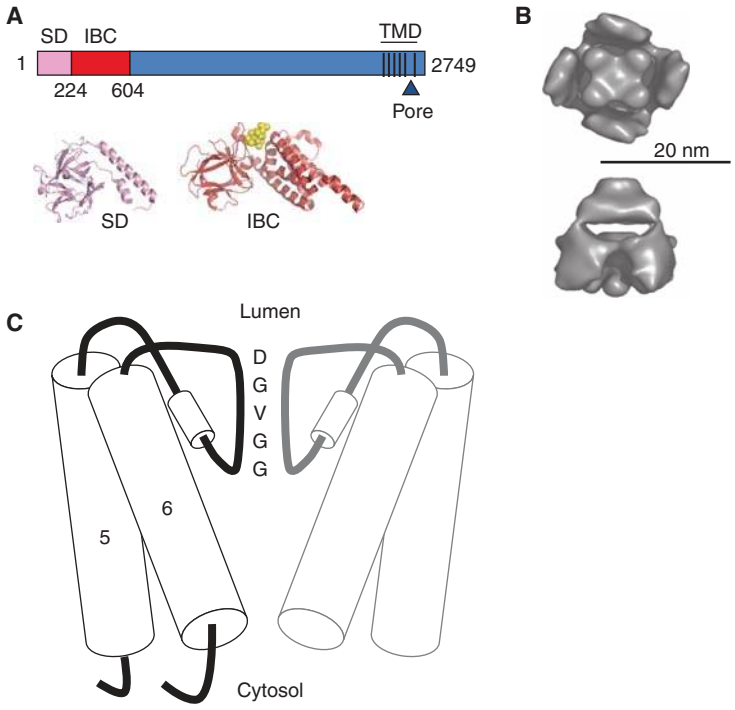

Figure 2. Major structural domains of IP_3R. (*A*) The three key regions defined by the primary sequence of a single IP_3R subunit are highlighted: the N-terminal with its SD and IBC, the C-terminal region with its six TMD and pore, and the large central region. Atomic structures of the SD (Bosanac et al. 2005) and IBC with IP_3 bound (Bosanac et al. 2002) are also shown. (*B*) Two views of the IP_3R derived from single particle analysis (da Fonseca et al. 2003) (*top*, from the cytosol; *bottom*, across the ER membrane with the ER lumen at the top). (*C*) A possible structure of the IP_3R pore, with a luminal selectivity filter and a constriction formed by the tepee-like structure of TMD6. Only two of the four IP_3R subunits are shown.

2002; Jiang et al. 2002a; da Fonseca et al. 2003; Hamada et al. 2003; Serysheva et al. 2003; Sato et al. 2004). These studies confirm the tetrameric state of IP_3R, but variability between the structures and their relatively low resolution (~30 Å) have, so far, limited any realistic interpretation of the structural basis of IP_3R activation (Taylor et al. 2004) (Fig. 2B). Whether structures of recombinant IP_3R will contribute to resolving this impasse remains to be seen (Wolfram et al. 2010).

There has been more progress with RyR, although only recently has the resolution of these structures (~30 Å) improved on that obtained for IP_3R. These structures of native RyR, and all three subtypes of recombinant RyR reveal a shape like a square mushroom with a very large, open cytoplasmic structure tethered to a much smaller TMD region (the stalk). At ~30 Å resolution, the structures of the three RyR subtypes are almost indistinguishable, and because they, like the three subtypes of IP_3R, share about 65% sequence identity, it seems reasonable to suppose that the 3D structures of all IP_3R are also likely to be similar to each other. These studies of RyR have identified positions of critical residues within the 3D structure, the sites to which accessory proteins bind, and conformational changes associated with opening of the pore (Orlova et al. 1996; Serysheva et al. 2005; Wang et al. 2007; Jones et al. 2008). Activation of RyR is associated with considerable changes in both the pore and cytoplasmic regions: The four corners of the latter dip down toward the SR, while the central region lifts away from it (Samso et al. 2009). It is noteworthy, in

the context of schemes for activation of IP_3R (see below), that large movements of some cytoplasmic domains of RyR1 appear to occur around hinges that link them to relatively immobile domains.

The highest resolution maps (~10Å), although still insufficient to map 3D structure to primary sequence, have come close to defining the likely secondary structure of the pore of RyR1 (Ludtke et al. 2005; Samso et al. 2005; Samso et al. 2009). This region appears to have six α-helices (Samso et al. 2009), consistent with models of RyR that suggest six TMD (Meur et al. 2007). Along the central axis, it has a luminal constriction (probably the selectivity filter, see below) and a tepee-like assembly of four inner helices (likely to be TMD6), with the apex pointing into the cytoplasmic structure. By analogy with MthK channels, this constriction may form the gate of the RyR. Kinking of the inner helix around a central Gly residue causes splitting of the tepee and thereby opening of the channel for MthK (Jiang et al. 2002b). One structure (Samso et al. 2009) is consistent with a similar mechanism operating for RyR1, but another structure (Ludtke et al. 2005) and mutagenesis of the critical Gly (G4863 in RyR1) (Wang et al. 2003) contradict it. These insights into the possible workings of the RyR pore are significant for IP_3R, because it is within the pore region (TMD5-6) that RyR and IP_3R share the greatest sequence similarity. We turn, therefore, to the pore of the IP_3R to explore its properties and structure.

All IP_3R (like all RyR) are cation channels with extremely large conductance, but only modest selectivity for Ca^{2+} over monovalent cations (permeability ratio, $P_{Ca}/P_K \sim 6$) (Williams et al. 2001; Foskett et al. 2007). The voltage-gated and store-operated Ca^{2+} channels that mediate Ca^{2+} entry across the plasma membrane are vastly more selective ($P_{Ca}/P_K > 1000$). In the ER, where most IP_3Rs are located, this lack of selectivity is unlikely to be a problem because Ca^{2+} is probably the only cation with an appreciable electrochemical gradient across the ER membrane. In effect, the ER Ca^{2+} pump (SERCA), by creating a steep Ca^{2+} concentration gradient across the ER membrane, assumes responsibility for determining which cations flow through an open IP_3R. Indeed, the K^+ permeability of IP_3R and RyR may facilitate rapid Ca^{2+} release by allowing K^+ to move into the ER to electrically compensate the efflux of Ca^{2+} (Gillespie and Fill 2008). The pore of the IP_3R, like that of RyR, is formed by the final pair of TMD (TMD5-6) and the luminal loop that links them from each of the four subunits (Ramos-Franco et al. 1999; Williams et al. 2001) (Fig. 2C). The loop includes a sequence (GGVGD in IP_3R) similar to that of the selectivity filter of K^+ channels (Balshaw et al. 1999), consistent with the idea that the overall architecture of the pore region may be broadly similar to that of K^+ channels (MacKinnon 2004). For both IP_3R and RyR, however, the pore must be larger and less-selective than for K^+ channels, and probably able to accommodate only one cation at a time (Williams et al. 2001). This model for the IP_3R pore, where TMD5 (the outer helix) and TMD6 (inner helix) cradle a short pore helix and selectivity filter (Fig. 2C), is consistent with mutagenesis of residues within this region affecting ion permeation (Boehning et al. 2001b; Dellis et al. 2006; Dellis et al. 2008; Schug et al. 2008), with biophysical evidence that the narrowest region of the pore lies close to the luminal entrance of the RyR (Williams et al. 2001) and with the intermediate resolution structures of the pore region of RyR1 (Samso et al. 2009). A conserved acidic residue (D2550 in IP_3R1) at the luminal end of the selectivity filter (Fig. 2C) contributes to the modest Ca^{2+} selectivity of IP_3R (Boehning et al. 2001b; Dellis et al. 2008) and RyR (Gao et al. 2000; Wang et al. 2005; Gillespie 2008), but the structural determinants of ion selectivity and permeation by IP_3R are otherwise poorly understood. The changes in pore structure that allow it to open are also minimally understood. Indeed, mutation of the conserved Gly within TMD6 of IP_3R (G2586 in IP_3R1), which might have been thought to provide the gating hinge (Samso et al. 2009), appears not to prevent IP_3 from opening IP_3R (Schug et al. 2008). In short, aside from knowing the regions of primary sequence that form the IP_3R pore (TMD5-6) and a rather vague notion that its structure perhaps resembles that of K^+ channels, we have only the most

rudimentary knowledge of the structural determinants of how the IP_3R pore opens and selects between ions.

The conformational changes in the IP_3R that lead to opening of its pore are initiated by IP_3 binding to the IP_3-binding core (IBC, residues 224–604 in IP_3R1) (Fig. 3A). Although IP_3 is the only endogenous ligand of the IBC, there are many synthetic agonists, all of which have structures equivalent to the equatorial 6-hydroxyl and the 4- and 5-phosphate groups of IP_3 (Fig. 3A) (Rossi et al. 2010). It is noteworthy that neither of the immediate products of IP_3 metabolism, IP_2 and IP_4, binds to the IBC; both metabolic pathways are therefore effective means of terminating activation of IP_3R by IP_3. An atomic structure of the IBC with IP_3 bound (Bosanac et al. 2002) shows IP_3 held within a clam-like structure in which the phosphate groups of IP_3 are coordinated by basic residues (Fig. 3A). The two sides of the clam, the α- and β-domains, form a network of interactions with the essential groups of IP_3. The 4-phosphate is hydrogen-bonded with residues in the β-domain, the 5-phosphate forms hydrogen bonds with residues predominantly in the α-domain, and the 6-hydroxyl interacts with the backbone of a residue within the α-domain. It is easy to imagine how these interactions with IP_3 might pull the α- and β-domains together, causing the clam to close in a manner similar to glutamate binding to ionotropic glutamate receptors (Mayer 2006). Structures of the IBC without IP_3 bound are urgently needed to assess this proposal, but two lines of evidence lend circumstantial support. First, the IBC adopts a more constrained structure when it binds IP_3 (Chan et al. 2007). Second, adenophostins, which are high-affinity agonists of IP_3R (Rossi et al. 2010), retain some activity after loss of the 3-phosphate (analogous to the 5-phosphate of IP_3), probably because their adenine moiety

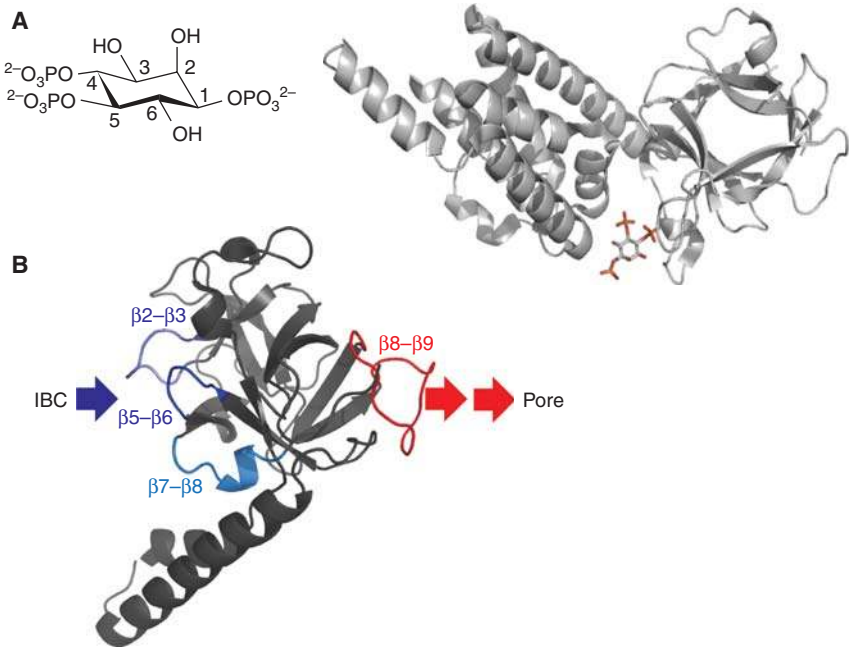

Figure 3. Initiation of IP_3R activation by IP_3. (*A*) The structure of IP_3, with its critical vicinal 4,5-bisphosphate and 6-hydroxyl groups, is shown alongside the structure of the IBC with IP_3 bound. The latter shows the 4- and 5-phosphates contacting the β- and α-domains, respectively (Bosanac et al. 2002), and thereby pulling the clam into a more closed state. (*B*) Structure of the SD (Bosanac et al. 2005) showing possible sites of interaction with the IBC and downstream domains through which it signals to the pore. See text for further details.

interacts strongly with a residue in the α-domain and thereby partially mimics the clam-closure that would otherwise require the 5-phosphate to bind to the α-domain (Sureshan et al. 2009). We envisage, therefore, that when IP_3 binds to the IBC, the essential vicinal phosphate groups through their contacts with the α- and β-domains effectively cross-bridge the two sides of the clam-like structure, causing it to close, and thereby initiate the processes that will culminate in opening of the pore.

It is worth commenting briefly on available antagonists of IP_3R because of their obvious value as experimental tools. There are no specific antagonists of IP_3R, although with appropriate caution some antagonists can yield useful insight (Michelangeli et al. 1995). Heparin is a competitive antagonist of IP_3 (Worley et al. 1987), although it is not membrane-permeant and, among many additional effects, it uncouples G-protein-coupled receptors from their G proteins (Dasso and Taylor 1991) and activates RyR (Ehrlich et al. 1994). 2-aminoethyl diphenylboronate (2-APB) is membrane-permeant and inhibits IP_3-evoked Ca^{2+} release without affecting IP_3 binding (Maruyama et al. 1997); its mechanism of action is unresolved. However, 2-APB also inhibits Ca^{2+} uptake and many other Ca^{2+} channels. It has recently aroused interest as a modulator of STIM and, therefore, store-operated Ca^{2+} entry (Goto et al. 2010). A screen of 2-APB analogues with selectivity for store-operated Ca^{2+} entry may yet also provide IP_3R-selective antagonists (Goto et al. 2010). Xestospongins, isolated from an Australian sponge, are high-affinity membrane-permeant inhibitors of IP_3-evoked Ca^{2+} release that do not affect IP_3 binding (Gafni et al. 1997), but they, too, have side effects (Solovyova et al. 2002). High concentrations of caffeine inhibit IP_3-evoked Ca^{2+} release (Parker and Ivorra 1991) without affecting IP_3 binding (Worley et al. 1987), but caffeine also stimulates RyR, inhibits cyclic nucleotide phosphodiesterases, and interferes with many Ca^{2+} indicators. Membrane-permeant peptide antagonists of IP_3R may provide another potential source of selective antagonists (Sun and Taylor 2008).

How IP_3 binding to the IBC leads to binding of Ca^{2+} to the IP_3R, and thereby opening of the pore, remains largely unknown, but it is clear that the suppressor domain (SD, residues 1-223 of IP_3R1), which is connected to the IBC by a flexible linkage (Chan et al. 2007), plays an essential role. The clearest evidence is that IP_3 binds to IP_3R without an SD, but it fails to open the pore (Uchida et al. 2003; Szlufcik et al. 2006). The name of this region derives from the observation that, although the SD itself is unlikely to make any direct contacts with IP_3, its presence decreases the affinity of IP_3R for IP_3 (Uchida et al. 2003). We have interpreted this effect to reflect the use of binding energy from the binding of IP_3 to the IBC to cause a conformational change within the SD. This interpretation gains considerable support from our analysis of partial agonists of the IP_3R (Rossi et al. 2009). The crux of our argument is that the energy provided by agonist binding drives both the conformational changes that lead to receptor activation and tighter binding of the ligand to its receptor. There is, therefore, a playoff between these two claims on the binding energy. Partial agonists, because they less effectively activate the receptor, divert more binding energy into stabilizing the binding, while full agonists evoke more substantial conformational changes; therefore, less binding energy remains to stabilize binding. Our results show that although full and partial agonists bind with similar affinities to the IBC, the SD causes the affinity of full agonists to decrease more than for partial agonists (Rossi et al. 2009). Quantitative analyses of these results lead to the conclusion that the most energetically costly conformational change in the IP_3R evoked by IP_3 occurs within its N-terminal (residues 1-604), and that these conformational changes pass entirely via the SD to the pore region (Rossi et al. 2009). We suggest, therefore, that the SD is the essential link between IP_3 binding to the IBC and the subsequent conformational changes that lead to opening of the pore. Without a structure of the entire N-terminal of the IP_3R, we can only speculate on the physical relationship between the IBC and SD, but our results with partial agonists and mutagenesis are

consistent with three exposed loops of the SD (β2–β3, β5–β6, and β7–β8, blue in Fig. 3B) being the most likely sites of interaction with the IBC (Rossi et al. 2009).

Remarkably, and despite their rather low sequence identities (~30%), the crystal structures of the SD from IP_3R1 (Bosanac et al. 2005) and of the analogous N-terminal regions from RyR1 and RyR2 (Amador et al. 2009; Lobo and Van Petegem 2009) are extremely similar. Several mutations associated with malignant hyperthermia and central core disease (RyR1) or catecholaminergic polymorphic ventricular tachycardia (RyR2), all of which impair the normal regulation of gating, are clustered in an exposed loop (β8–β9) of the N-terminal of RyR (Amador et al. 2009). Furthermore, and consistent with the N-terminal of the RyR mediating essential interdomain interactions, a peptide derived from this region causes RyR2 to open spontaneously, apparently by uncoupling an interaction between the endogenous loop and a central region of the RyR that includes residues 2460–2495 (Oda et al. 2005; Tateishi et al. 2009). In light of the conservation of structure between IP_3R and RyR, it is tempting to speculate that the same loop in the SD of the IP_3R (β8–β9, red in Fig. 3B) may mediate transfer of conformational changes onward toward the pore. Co-immunoprecipitation studies have suggested an interaction between the N-terminal of IP_3R1 (most likely the SD) and the pore region of an adjacent subunit (Boehning and Joseph 2000a), perhaps mediated by the cytosolic loop linking TMD4 to TMD5 (Schug and Joseph 2006). An attractive possibility, therefore, is that the SD (perhaps its β8-β9 loop) interacts directly with the short cytosolic helix linking TMD4 and TMD5, and thereby gates the pore (Schug and Joseph 2006; Rossi et al. 2009). Such an interaction would require that the SD comes very close to the pore, but the exact location of the SD within the 3D structure of either the IP_3R or RyR is unknown. The N-terminal of the RyR probably lies within the clamp region at the periphery of the large square cytoplasmic structure (Wang et al. 2007), and it does change shape during RyR activation (Samso et al. 2009). Yet, in this location the N-terminal is too far from the pore to interact directly with the TMD4-5 loop, consistent perhaps with evidence that in RyR the N-terminal may interact directly with a neighboring domain that includes residues from the central part of the primary sequence (Wang et al. 2007). These observations and the evidence that the uncoupling peptide derived from the N-terminal of RyR2 appears to interact with residues remote from the pore (Oda et al. 2005; Tateishi et al. 2009), suggest that the links between the SD and pore may, at least for RyR, be indirect.

In summary, we suggest that IP_3R activation is initiated when IP_3 binds to the IBC, and perhaps thereby causes closure of its clam-like structure. That conformational change, which must also initiate the events that allow Ca^{2+} to bind to a stimulatory site, is passed to the rest of the IP_3R entirely via the SD. The location of that Ca^{2+}-binding site and, therefore, the structural links between it and the SD, are unresolved. We speculate that one face of the SD interacts directly with the IBC, and the opposite face interacts with the structure through which conformational changes pass to the pore. The pore is a relatively nonselective, large-conductance cation channel formed by the tetrameric assembly of the TMD5-6 regions of each subunit. Its structure is unresolved but likely to be broadly similar to K^+ channels with a selectivity filter and gate at opposite ends of its membrane-spanning structure.

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