

Structure and Function of IP₃ Receptors

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Inositol 1,4,5-trisphosphate receptors (IP₃Rs), by releasing Ca²⁺ from the endoplasmic reticulum (ER) of animal cells, allow Ca²⁺ to be redistributed from the ER to the cytosol or other organelles, and they initiate store-operated Ca²⁺ entry (SOCE). For all three IP₃R subtypes, binding of IP₃ primes them to bind Ca²⁺, which then triggers channel opening. We are now close to understanding the structural basis of IP₃R activation. Ca²⁺-induced Ca²⁺ release regulated by IP₃ allows IP₃Rs to regeneratively propagate Ca²⁺ signals. The smallest of these regenerative events is a Ca²⁺ puff, which arises from the nearly simultaneous opening of a small cluster of IP₃Rs. Ca²⁺ puffs are the basic building blocks for all IP₃-evoked Ca²⁺ signals, but only some IP₃ clusters, namely those parked alongside the ER–plasma membrane junctions where SOCE occurs, are licensed to respond. The location of these licensed IP₃Rs may allow them to selectively regulate SOCE.

Inositol 1,4,5-trisphosphate receptors (IP₃Rs) are expressed in most animal cells, including single-celled protozoa (Prole and Taylor 2011). They mediate release of Ca²⁺ from intracellular stores, primarily the endoplasmic reticulum (ER) (Berridge 1993) and Golgi apparatus (Pizzo et al. 2011; Wong et al. 2013; Rodriguez-Prados et al. 2015). IP₃Rs are also expressed in the nuclear envelope and nucleoplasmic reticulum (Echevarría et al. 2003), where they may selectively generate nuclear Ca²⁺ signals, although cytosolic Ca²⁺ signals also invade the nucleoplasm (Bading 2013). IP₃R-mediated Ca²⁺ fluxes across ER membranes increase the cytosolic Ca²⁺ concentration ([Ca²⁺]_c), and when these signals occur close to other organelles, mitochondria (Csordas et al. 2018) or lysosomes (Lopez Sanjurjo et al. 2013; Garrity et al. 2016; Atakpa et al. 2018), for example, they allow

their low-affinity uptake systems to resequester the Ca²⁺. The accompanying decrease in ER luminal Ca²⁺ concentration is also important because it activates stromal interaction molecule 1 (STIM1), which then accumulates at ER–plasma membrane (PM) junctions. Within these narrow junctions, STIM1 in the ER membrane interacts directly with Orai1, which is a hexameric Ca²⁺ channel in the PM (Hou et al. 2012; Yen and Lewis 2018), causing it to open (Prakriya and Lewis 2015). The resulting store-operated Ca²⁺ entry (SOCE) is almost universally associated with IP₃-evoked Ca²⁺ release. Hence, in response to the many extracellular stimuli that evoke IP₃ formation, IP₃Rs allow Ca²⁺ to be rapidly redistributed from the ER to the cytosol or other organelles and, by controlling the Ca²⁺ content of the ER, IP₃Rs control Ca²⁺ flowing into the cell through SOCE (Fig. 1).

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D.L. Prole and C.W. Taylor

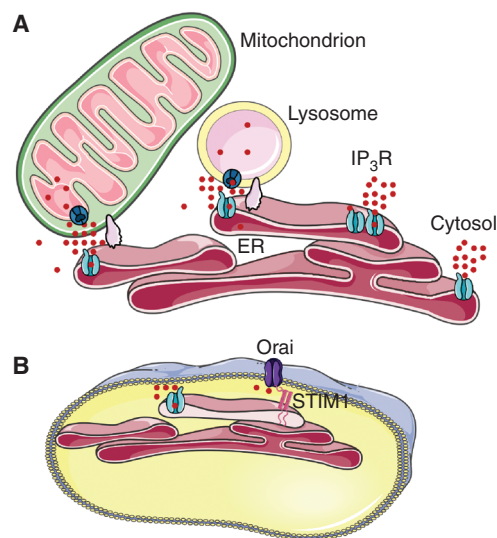


Figure 1. IP₃ receptors deliver Ca²⁺ to the cytosol and organelles. (A) By releasing Ca²⁺ from the endoplasmic reticulum (ER), IP₃Rs can deliver Ca²⁺ to the cytosol, to other IP₃Rs to ignite regenerative signals, or to the close appositions (membrane contact sites, supported by scaffold proteins) between the ER and other organelles. The latter include mitochondria and lysosomes, which can then accumulate Ca²⁺ via their low-affinity uptake systems from the high local Ca²⁺ concentration provided by IP₃Rs. (B) Loss of Ca²⁺ from the ER also activates STIM1, which then binds to Orai at ER–plasma membrane (PM) junctions to initiate store-operated Ca²⁺ entry (SOCE).

The cytosolic Ca²⁺ signals evoked by IP₃Rs or SOCE regulate diverse physiological responses, including exocrine secretion (Futatsugi et al. 2005), gluconeogenesis (Wang et al. 2012), embryological development (Kume et al. 1997; Uchida et al. 2010), transcription (Kar et al. 2012), nerve growth (Takei et al. 1998), and migration (Wei et al. 2009). The ability of IP₃Rs to deliver Ca²⁺ to the mitochondrial uniporter (MCU) allows regulation of oxidative phosphorylation (Cardenas et al. 2010, 2016) and apoptosis (La Rovere et al. 2016), and Ca²⁺ delivery to lysosomes may allow them to accumulate Ca²⁺, which regulates their activities (Xu and Ren 2015). Dysregulation of IP₃Rs is implicated in human diseases including Huntington’s disease, Alzheimer’s disease, amyotrophic lateral sclerosis, ataxias, autism, and cancer (Berridge 2016).

Despite the importance of IP₃Rs in both normal physiology and disease, the only known antagonists of IP₃Rs (heparin, caffeine, and Xestospongin) lack specificity (Saleem et al. 2014). There is a pressing need for selective, membrane-permeant IP₃R antagonists.

Ca²⁺-binding sites, which include those on the proteins that decode Ca²⁺ signals, are so abundant in cytosol that only some 1% of Ca²⁺ entering the cytosol remains free, although that buffering capacity does vary widely between cell types (Schwaller 2012). A cytosolic Ca²⁺ ion therefore spends the most time held by a buffer with which it moves more slowly than when free, and when it dissociates it is likely to diffuse freely for only a brief interval before it is recaptured by another buffer. Schwaller (2012) has suggested an apt analogy with Velcro to describe this behavior, which ensures that cytosolic Ca²⁺ diffuses slowly (Allbritton et al. 1992). This is an important feature that allows Ca²⁺ to linger at open channels and underpins its ability to serve as a local messenger. Hitherto, it has been assumed that IP₃ diffuses freely in cytosol, based largely on measurements from cytoplasmic extracts of *Xenopus* oocytes in a rightly influential paper (Allbritton et al. 1992). Hence, the widely promulgated assumption has been that Ca²⁺ is a local messenger, while IP₃ is a global messenger. However, IP₃Rs in *Xenopus* oocytes are concentrated in a narrow rim beneath the PM, whereas they are distributed throughout the cytoplasm of more typical cells (Thillaiappan et al. 2017). The cytoplasmic density of IP₃Rs considered alongside their affinity for IP₃ and the necessity for an IP₃R to bind four molecules of IP₃ before it can open (Alzayady et al. 2016) suggest that IP₃Rs may, and prior to their activation, appreciably buffer IP₃ (Taylor and Konieczny 2016). Estimates of IP₃ diffusion in SH-SY5Y neuroblastoma cells, derived from measuring the extent to which IP₃ focally released from a caged precursor spreads to initiate local Ca²⁺ signals, have elegantly confirmed that diffusion of IP₃ in cells (diffusion coefficient, $D \sim 10 \mu\text{m}^2/\text{sec}$) is ~ 30 -fold slower than expected (Dickinson et al. 2016) and comparable to Ca²⁺ diffusion ($D = 13\text{--}65 \mu\text{m}^2/\text{sec}$) (Allbritton et al. 1992). This suggests that both intracellular messengers,

IP₃ and Ca²⁺, can act locally within the confines of a typical cell (Dickinson et al. 2016). The activities of many cells are coordinated by Ca²⁺ waves that spread between cells (Leybaert and Sanderson 2012). Diffusion of IP₃ through intercellular gap junctions is one means by which such Ca²⁺ waves are thought to propagate, but that idea was influenced by the assumption that IP₃ diffusion is unhindered (Leybaert 2016). The discovery that IP₃ diffuses slowly may require reappraisal of current thinking on how intercellular Ca²⁺ waves propagate and it invites speculation that there may be “highways” between cells wherein IP₃ buffering is reduced to facilitate faster intercellular diffusion.

In a contribution to the first edition of this collection, we reviewed the history of IP₃Rs (Taylor and Tovey 2012), noting that it was entwined with that of ryanodine receptors (RyRs), the close cousins of IP₃Rs. The cross fertilization between studies of these two major families of intracellular Ca²⁺ release channels, with their many structural and functional similarities (Seo et al. 2012; des Georges et al. 2016), continues to provide important insight. That interplay will again be apparent in this review. We focus on recent progress toward understanding the structural basis of IP₃R activation, evidence that IP₃Rs are regulated by many additional proteins, and the organization of IP₃Rs within ER membranes and the implications of that for SOCE. Other reviews provide readers with broader overviews (Foskett et al. 2007), historical perspectives (Berridge 2005; Rossi and Taylor 2019), and more focused considerations of IP₃Rs and disease (Berridge 2016; Hisatsune and Mikoshiba 2017; Egorova and Bezprozvanny 2018), their regulation by proteolysis (Wang and Yule 2018) and other signals (Prole and Taylor 2016; Taylor 2017), the evolution of IP₃Rs (Alzayady et al. 2015), and relationships between SOCE and IP₃Rs (Taylor and Machaca 2019; Thillaiappan et al. 2019). We begin with a short overview of IP₃Rs.

IP₃ RECEPTORS ARE REGULATED BY IP₃ AND Ca²⁺

Vertebrate genomes encode subunits for three closely related IP₃R subunits (IP₃R1–3), which

assemble into homo- and heterotetrameric channels. The subunits are enormous (~2700 residues), such that IP₃Rs and RyRs (which are even larger, 4 × ~5000 residues/RyR) are the largest known ion channels. The IP₃R subtypes differ in their patterns of expression between tissues (Taylor et al. 1999) and perhaps in their subcellular distributions (Vervloessem et al. 2015), they have different affinities for IP₃ (IP₃R2 > IP₃R1 > IP₃R3) (Iwai et al. 2007), they differ in their associations with other proteins and in their modulation by additional signals (Prole and Taylor 2016), they appear to differ in their capacity to sustain oscillatory Ca²⁺ signals (Miyakawa et al. 1999; Wang and Yule 2018), and the functional consequences of perturbing IP₃Rs differ for the different subtypes (Hisatsune and Mikoshiba 2017). Despite the differences, the core functional properties of all IP₃Rs are similar and so too are their structures, consistent with the sequence conservation (~70%) between subtypes (Fan et al. 2015, 2018; Paknejad and Hite 2018). All IP₃Rs form large-conductance cation channels with relatively weak selectivity for Ca²⁺ over K⁺ (P_{Ca}/P_K ~ 7) (Foskett et al. 2007). The large conductance, which allows a single IP₃R to conduct ~10⁵ Ca²⁺/sec or 1000 Ca²⁺ ions for each 10-msec opening (Vais et al. 2010), is important because it permits small numbers of IP₃Rs to rapidly deliver large local Ca²⁺ signals to the cytosol. The second feature common to all IP₃Rs, although historically it has spawned some controversy, is their biphasic regulation by [Ca²⁺]_c. The activity of all IP₃Rs is enhanced by modest increases in [Ca²⁺]_c and inhibited by more substantial increases (Iino 1990; Bezprozvanny et al. 1991; Foskett et al. 2007). Whether IP₃Rs are also regulated directly by Ca²⁺ within the ER lumen remains a contentious and unresolved issue (Irvine 1990; Nunn and Taylor 1992; Vais et al. 2012).

Activation of IP₃Rs requires binding of both IP₃ to all four of its subunits (Alzayady et al. 2016) and Ca²⁺ binding (Finch et al. 1991; Marchant and Taylor 1997). The simplest scheme envisages two Ca²⁺-binding sites associated with the IP₃R (Marshall and Taylor 1994). Biophysical analyses tentatively suggest that the stimulatory Ca²⁺-binding site may be closer to

D.L. Prole and C.W. Taylor

the pore than the inhibitory site (Vais et al. 2012). Different schemes have been proposed to explain the interaction between IP₃ and Ca²⁺, with IP₃ proposed to regulate only the inhibitory Ca²⁺-binding site (reducing its affinity for Ca²⁺) (Mak et al. 1998; Vais et al. 2012) or both the inhibitory (reducing its Ca²⁺ affinity) and stimulatory (increasing its Ca²⁺ affinity) sites (Marchant and Taylor 1997; Adkins and Taylor 1999). Whatever the detailed mechanism, the outcome is that IP₃ primes IP₃Rs to respond to stimulation by Ca²⁺, by either divorcing the stimulatory and inhibitory effects, or by directly promoting Ca²⁺ binding to the stimulatory site (Fig. 2A,B). This interplay has important implications because it allows IP₃Rs, in the presence of IP₃, to propagate Ca²⁺ signals regeneratively by Ca²⁺-induced Ca²⁺ release (CICR) (Fig. 2C). We return to this feature later, but first we consider progress toward understanding the structural basis of how IP₃ and Ca²⁺ binding together lead to opening of a large-conductance channel through which Ca²⁺ can leave the ER.

HOW TO OPEN AN IP₃ RECEPTOR

How does IP₃ binding to a site, the IP₃-binding core (IBC), located ~7 nm from the constriction within the closed channel, lead to channel open-

ing? Progress toward answering this question has come from high-resolution crystal structures of the amino-terminal region of the IP₃R, which includes the IBC (Bosanac et al. 2002, 2005; Lin et al. 2011; Seo et al. 2012) and of the entire cytosolic region (Hamada et al. 2017). These analyses capture structures of only one subunit of the tetrameric IP₃R. Cryoelectron microscopy (cryo-EM) structures of IP₃R1 (Fan et al. 2015, 2018) and of IP₃R3 with and without IP₃ and Ca²⁺ (Paknejad and Hite 2018) capture different states of the complete protein. Structural analyses of RyR fragments (Amador et al. 2009; Tung et al. 2010; Kimlicka et al. 2013; Liu et al. 2015) and of complete structures of RyR1 and RyR2 in various states (Efremov et al. 2015; Yan et al. 2015; Zalk et al. 2015; des Georges et al. 2016; Peng et al. 2016) also provide insight into the workings of IP₃Rs.

The structure of the IP₃R resembles a square mushroom, most of which (~90%) is in the cytosol (Fig. 3A; Fan et al. 2015; Paknejad and Hite 2018). Most of the stalk is embedded in the ER membrane and the cap, with a diameter of ~25 nm, extends ~13 nm into the cytosol. The large size of IP₃Rs is relevant, not only for the opportunities it provides for cryo-EM analysis and the technical challenges it presents to crystallographers and molecular biologists, but also

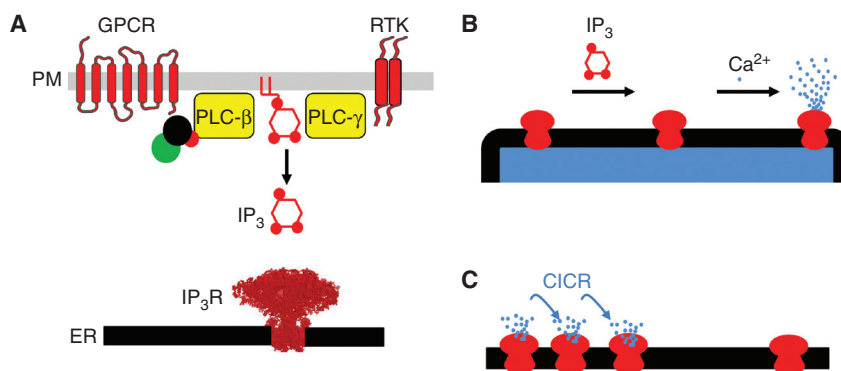


Figure 2. IP₃ receptors are stimulated by IP₃ and Ca²⁺. (A) Many receptors, including G-protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs), can stimulate phospholipase C (PLC), leading to production of IP₃, which then binds to IP₃Rs in the endoplasmic reticulum (ER). (B) IP₃ binding to IP₃R primes them to bind Ca²⁺, which then stimulates the channel to open, allowing Ca²⁺ to flow out of the ER. (C) This dual regulation of IP₃Rs by IP₃ and Ca²⁺ allows them to mediate regenerative signals propagated by Ca²⁺-induced Ca²⁺ release (CICR). PM, Plasma membrane.

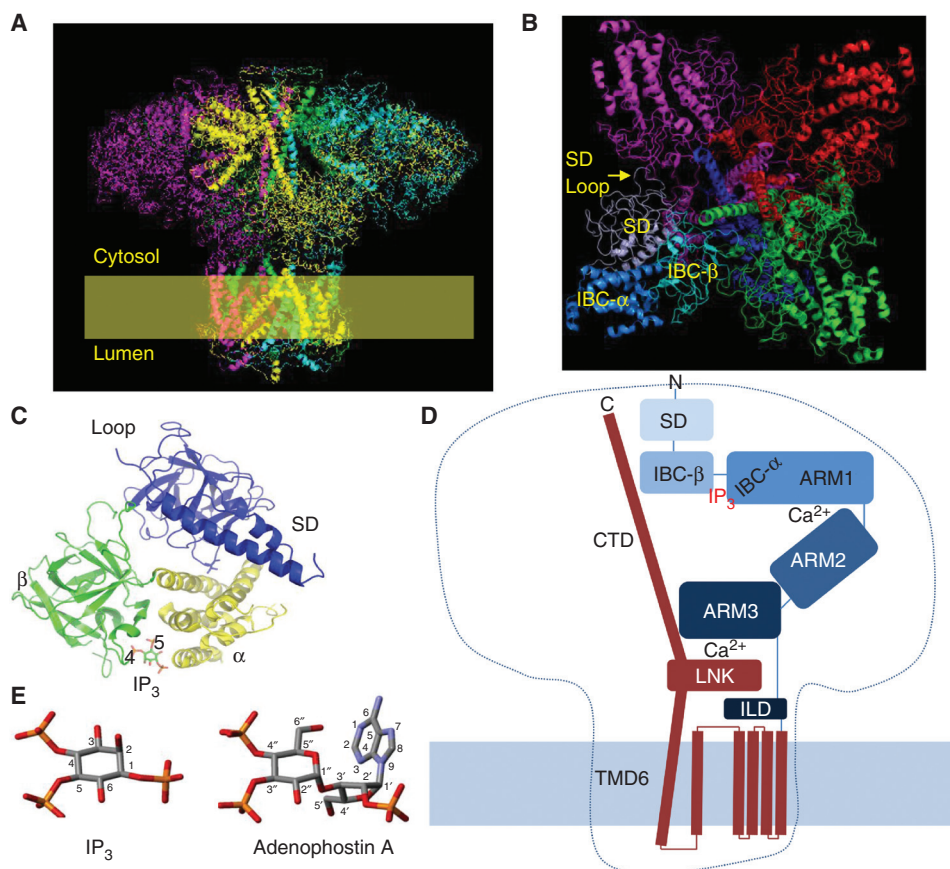


Figure 3. IP₃ receptor structure. (A) Cryoelectron microscopy (cryo-EM) of IP₃R1 shows its tetrameric mushroom-like structure. (From Fan et al. 2015; adapted, with permission, from Springer Nature © 2015.) Subunits are color-coded. A similar structure has been reported for IP₃R3 (Paknejad and Hite 2018). (B) View from the cytosol. (C) The amino-terminal region of each IP₃R subunit comprises the suppressor domain (SD) with the “hot spot” loop through which it contacts an adjacent subunit; and the IP₃-binding core (IBC), with its α and β domains. The essential 4- and 5-phosphates of IP₃ interact predominantly with residues on the inner surface of the β and α domains, respectively, to trigger partial closure of clam-like IBC. (D) Schematic representation showing a single IP₃R subunit, highlighting the IBC, where IP₃ binds, two Ca²⁺-binding sites at interfaces between ARM1 and ARM2 domains, and between the LNK and ARM3 domains (Paknejad and Hite 2018). The α -helical rod (carboxy-terminal domain [CTD]) extending from LNK to the cap of the mushroom was resolved in structures from one laboratory (Fan et al. 2015, 2018), but not in the structures determined by another laboratory (Paknejad and Hite 2018). It is clear that conformational changes initiated by IP₃ binding must pass through a critical nexus formed by the LNK (from the pore region) and intervening lateral domains (ILDs) (from the cytosolic domain). (E) Structures of IP₃ and adenophostin A, showing how the latter has structures equivalent to the essential 4- and 5-phosphates of IP₃.

for its cellular functions. IP₃Rs may, for example, be too large to fit within the narrow ER–PM junctions where SOCE occurs (Thillaiappan et al. 2017), but their large size allows IP₃Rs to accrete accessory proteins (see below) and it may allow them to more effectively deliver Ca²⁺ to

the surface of juxtaposed organelles, like mitochondria or lysosomes (Fig. 1A).

Within the ER membrane, there are probably 24 transmembrane domains (TMDs), six contributed by residues toward the carboxy-terminal end of each IP₃R subunit, although a

D.L. Prole and C.W. Taylor

recent report suggests the possible presence of two additional TMDs between TMD1 and TMD2 in IP₃Rs (Fan et al. 2018; Paknejad and Hite 2018) and perhaps also in RyR (des Georges et al. 2016). The structure of the IP₃R transmembrane region is similar in RyRs and, to a lesser extent, in voltage-gated cation channels, most of which have six TMDs per subunit. The ion-conducting path is lined by the four tilted TMD6 helices, which twist around each other. At the luminal end, there is a short (~1 nm) “selectivity filter” within which conserved backbone carbonyls may form a cation-binding site, allowing hydrated cations to pass in single file. The selectivity filter, its supporting pore-loop helix and a flexible luminal loop are all formed by residues linking TMD5 to TMD6. Near the cytosolic end of TMD6, a narrow hydrophobic constriction blocks movement of ions in the closed channel. Minimally, the hydrophobic side chains of these residues (Phe²⁵⁸⁶ and Ile²⁵⁹⁰ in IP₃R1) must move for the pore to open, but there may also be changes around the pore helix to displace a positively charged residue (His²⁵⁴¹) that might otherwise impede cation movements (Fan et al. 2015). Opening of RyR1 is associated with splaying and bowing of TMD6, such that the occluding hydrophobic side chain is displaced (des Georges et al. 2016). A similar mechanism may open the IP₃R pore (Fan et al. 2018). TMD6 extends well beyond the ER membrane (~1.5 nm) and then terminates in a short α -helical bundle (the linker, LNK) that includes a Zn²⁺-finger motif and aligns parallel with the ER membrane (Fan et al. 2015; Paknejad and Hite 2018). The functional significance of the Zn²⁺-finger is unknown. Hence, structures formed by the TMD5-6 loop guard the luminal entrance to the pore, while the cytosolic exit is formed by the extended TMD6. Each of these regions is enriched in negatively charged residues that probably contribute to the cation selectivity of the IP₃R.

IP₃ binding to the clam-like IBC initiates IP₃R activation (Fig. 3B,C). The IBC is located toward the amino terminal of the primary sequence of each subunit, and comprises two domains (α and β), with the pocket between them providing the positively charged residues that

interact with IP₃ (Bosanac et al. 2002; Seo et al. 2012; Paknejad and Hite 2018). All four subunits must bind IP₃ before the channel can open (Alzayady et al. 2016). It is less clear whether all stimulatory Ca²⁺-binding sites must be occupied for channel opening (Vais et al. 2012). The amino-terminal region forms a triangular structure at the top of the mushroom, with the suppressor domain (SD) (residues 1–223; also known as β -trefoil domain 1 [BTF1]) and IBC- β (BTF2) lining the cytosolic exit at the top of the IP₃R. The subunits interact through a loop within the SD that contacts IBC- β of a neighboring subunit (Fig. 3B,C; Seo et al. 2012; Fan et al. 2015; Hamada et al. 2017; Paknejad and Hite 2018). Mutations within this “hot spot” loop disrupt gating of IP₃R (Yamazaki et al. 2010) and RyR (Amador et al. 2009). IBC- α sits behind the β -trefoil structures at the tip of a series of largely α -helical domains (ARM 1–3) that extend in a boomerang-like shape to meet the LNK domain (Fig. 3A,D).

The two critical phosphate groups of IP₃ (P-4 and P-5) interact predominantly with basic residues (Arg and Lys) lining opposing sides of the IBC clam: P-4 with IBC- β and P-5 with IBC- α (Fig. 3C; Bosanac et al. 2002). These interactions allow IP₃ to partially close the clam-like IBC (Lin et al. 2011; Seo et al. 2012; Paknejad and Hite 2018), and they elegantly rationalize the long-established conclusion that all known agonists of IP₃Rs have structures equivalent to the 4- and 5-phosphates of IP₃. The structures also demonstrate how endogenous dephosphorylation of IP₃ to 1,4-IP₂ effectively terminates Ca²⁺ signaling. The importance of the clam closure is reinforced by results with adenophostin A analogs. Adenophostin A is a fungal product with 10-fold greater affinity for IP₃Rs than IP₃, and with structures, its 3''- and 4''-phosphates, equivalent to the 5- and 4-phosphates of IP₃, respectively (Fig. 3E; Rossi et al. 2010; but see Fan et al. 2018). Whereas loss of the 5-phosphate group from IP₃ abolishes activity, loss of the equivalent phosphate from adenophostin A (3''-phosphate) leaves some residual activity because the adenine moiety of adenophostin A can interact with IBC- α and so presumably mediate clam closure in the absence of the usual

phosphate group (Sureshan et al. 2009). Closure of the clam-like IBC by IP₃ binding is linked to IP₃R gating through the SD, but the exact sequence of conformational changes is unresolved. It may be that movement of IBC- α relative to a firmly anchored IBC- β /SD reorients the ARM domains (Paknejad and Hite 2018), or it may be that tethering of IBC- α to the SD causes reorientation of the SD and disruption of intersubunit interactions (Li et al. 2009; Seo et al. 2012; des Georges et al. 2016). It is, however, clear that IP₃-evoked conformational changes must pass through the contacts between ARM3 and LNK domains to reach the occluded pore (Fig. 3D).

ARM3 terminates in the intervening lateral domain (ILD), which sits between the cytosolic structures and the TMDs. ILD runs largely parallel to the ER membrane, and comprises two β strands (which lie immediately beneath ARM3) followed by a helix-turn-helix motif that links to TMD1 (Fig. 3D). The LNK domain (an extension of TMD6) is positioned between the β - and α -helical components of the ILD. Hence, interleaved structures formed by extensions of ARM3 (ILD) and TMD6 (LNK) form a critical nexus between the cytosolic region and the pore of the IP₃R. Mutations within ILD disrupt IP₃R function (Hamada et al. 2017), and the LNK domain contributes a conserved residue to a Ca²⁺-binding site at the base of the ARM3 domain (Fig. 3D; Paknejad and Hite 2018). This Ca²⁺-binding site, formed by residues at the interface of the cytosolic (base of ARM3) and pore (LNK domain) regions, is absolutely conserved in RyRs and IP₃Rs (des Georges et al. 2016). We note that in both RyR (Glu⁴⁰³² in RyR1) (Du and MacLennan 1998) and IP₃R1 (Glu²¹⁰⁰) (Miyakawa et al. 2001), a conserved glutamate was proposed to contribute to the stimulatory Ca²⁺-binding site. Indeed, and somewhat perplexingly, mutation of this residue affected both stimulation and inhibition of IP₃Rs by Ca²⁺ (Miyakawa et al. 2001). It is now clear from structural analyses that these conserved glutamates do not coordinate Ca²⁺ in either RyR (des Georges et al. 2016) or IP₃R (Paknejad and Hite 2018). It is equally clear that the EF-hand domain of RyR, which projects from a structure equivalent to ARM3 of the IP₃R, is absent from IP₃Rs (Fan et al.

2015), and nor does the EF-hand provide the essential Ca²⁺ regulation of RyRs (Guo et al. 2016). Hence, the conserved Ca²⁺-binding site at the interface between the cytosolic (ARM3) and channel domains (LNK) suggests an appealing, but untested, link between Ca²⁺ and gating of the IP₃R, namely that IP₃ binding stabilizes this Ca²⁺-binding site and Ca²⁺ binding to it then leads to opening of the pore. This proposal also aligns with the suggestion that the stimulatory Ca²⁺-binding site may be close to the pore (Vais et al. 2012). The high-resolution structure of IP₃R3 recently identified another Ca²⁺-binding site, which is also formed by residues provided by different domains across an interface between them (ARM1 and ARM2) (Paknejad and Hite 2018). It is not yet clear how (or whether) either of these Ca²⁺-binding sites relates to stimulation and inhibition of IP₃Rs by cytosolic Ca²⁺. It is, however, intriguing that both sites are formed by residues contributed by different domains, consistent with IP₃-evoked rigid-body domain movements influencing whether Ca²⁺ binds to the sites.

Recent progress has brought us close to seeing how IP₃ binding to the IBC causes pore residues some 7 nm away to move and allow Ca²⁺ to pass from the ER lumen to the cytosol (Fan et al. 2018; Paknejad and Hite 2018). IP₃ initiates IP₃R activation by causing closure of the IBC. That conformational change must then pass onward through a critical nexus formed between the cytoplasmic and pore domains at the ILD–LNK complex. Since IP₃ primes IP₃Rs to bind Ca²⁺, which then triggers channel opening (Fig. 2B; Adkins and Taylor 1999), we speculate that an intervening step between IP₃ binding to the IBC and pore opening involves rearrangement of Ca²⁺-binding sites at the ARM1–ARM2 interface or at the LNK–ARM3 nexus. The conformational changes evoked by Ca²⁺ binding must then pass through the ILD–LNK complex to cause movement of a hydrophobic residue in TMD6 and allow opening of the pore (Fig. 3D).

IP₃ RECEPTORS AS SIGNALING HUBS

IP₃ and Ca²⁺ are the essential regulators of IP₃R gating (Fig. 2), but many intracellular signals,

D.L. Prole and C.W. Taylor



including ATP (Wagner and Yule 2012), cAMP (Taylor 2017), H^+ (Worley et al. 1987), NADH (Kaplin et al. 1996), and the redox state (Joseph 2010; Joseph et al. 2018), can modulate this regulation. IP_3 Rs can also be regulated by covalent modifications, including phosphorylation, controlled by more than a dozen protein kinases and phosphatases, ubiquitination (Wojcikiewicz 2018), transglutaminase-mediated cross-linking of Gln-Lys residues (Hamada et al. 2014), and perhaps nitrosylation (Pan et al. 2008). Proteolysis of IP_3 Rs by caspase-3 (Hirota et al. 1999) or calpains (Magnusson et al. 1993) may contribute to their degradation. But after limited proteolysis, IP_3 R fragments remain associated as a functional channel. Intriguingly, native and cleaved IP_3 Rs respond differently, suggesting that proteolysis can provide more subtle regulation than merely down-regulating IP_3 Rs (Alzayady et al. 2013; Wang et al. 2017).

More impressive still is the huge array of proteins that associate with IP_3 Rs (Fig. 4A; Prole and Taylor 2016). These proteins, which may associate with cytosolic or luminal parts of the IP_3 R, can regulate the distribution of IP_3 Rs (Geyer et al. 2015), their affiliation with signaling pathways that deliver IP_3 (Tu et al. 1998) or cAMP (Tovey et al. 2008) to IP_3 Rs, the sensitivity of Ca^{2+} release to IP_3 and Ca^{2+} , and they may allow IP_3 Rs to deliver Ca^{2+} to specific decoding proteins (Fig. 4B; Szabadkai et al. 2006). Many of these modulatory influences are likely to be context specific, determined, for example, by IP_3 R subtype, cell type, and perhaps contingent on interactions between modulators (Ivanova et al. 2014; Prole and Taylor 2016). IP_3 R-binding protein released with IP_3 (IRBIT), for example, is a protein that competes with IP_3 for binding to the IBC, but it does so only after IRBIT phosphorylation (Ando et al. 2014).

Modulatory proteins also provide links between IP_3 Rs and human diseases, additional to those arising from loss or mutation of IP_3 Rs (Berridge 2016; Casey et al. 2017; Hisatsune and Mikoshiba 2017; Terry et al. 2018). The mutant forms of Huntingtin associated with Huntington's disease, mutant ataxins associated with spinocerebellar ataxias, and mutant presenilins associated with inherited forms of Alzheimer's

disease, for example, have each been reported to enhance IP_3 -evoked Ca^{2+} signals (Chen et al. 2008; Cheung et al. 2008, 2010; Liu et al. 2009; Egorova and Bezprozvanny 2018).

IP_3 R-evoked Ca^{2+} signals may also be targets for cancer therapeutics (Vervloessem et al. 2018). Transfer of Ca^{2+} from the ER to mitochondria via IP_3 Rs stimulates mitochondrial ATP production, but excessive Ca^{2+} transfer triggers apoptosis (Cardenas et al. 2010). ER-mitochondria Ca^{2+} transfer can thereby promote cell survival or death, according to the magnitude of the transfer. It has been suggested that tumor cells are "addicted" to ER-mitochondria Ca^{2+} transfer and so particularly susceptible to its inhibition because they lack the robust, protective autophagy response of normal cells (Cardenas et al. 2016). Here, inhibition of Ca^{2+} transfer to mitochondria might provide an opportunity to selectively kill cancer cells by necrosis (Cardenas et al. 2016). Conversely, exaggerating the transfer of Ca^{2+} from ER to mitochondria can trigger apoptosis. The tumor suppressors, Bap1 (Bononi et al. 2013) and PTEN (Kuchay et al. 2017), achieve this by protecting IP_3 Rs from proteosomal degradation, allowing sustained Ca^{2+} transfer to mitochondria and enhanced sensitivity to stimuli that promote apoptosis. The pro-apoptotic protein, Bok, achieves the same effect by protecting IP_3 Rs from cleavage by caspase (Schulman et al. 2013). Other anti-apoptotic members of the Bcl-2 family of proteins (e.g., Bcl-2, Bcl-XL) are also proposed to influence ER-mitochondrial Ca^{2+} transfer, and thereby apoptosis, but by regulating the activity, rather than the expression, of IP_3 Rs (discussed in Vervloessem et al. 2018). These interactions are now attracting interest as potential therapeutic targets in cancer.

Most accessory proteins that affect IP_3 -evoked Ca^{2+} release appear to do so indirectly by influencing IP_3 or Ca^{2+} binding or the interplay between them. However, a few proteins, including $G\beta\gamma$, which may mimic IP_3 (Zeng et al. 2003), and the Ca^{2+} -binding proteins, CIB1 (Ca^{2+} - and integrin-binding protein) (White et al. 2006) and CaBP1 (Yang et al. 2002), have been claimed to reversibly gate IP_3 Rs directly, bypassing the need for IP_3 . However, the sugges-

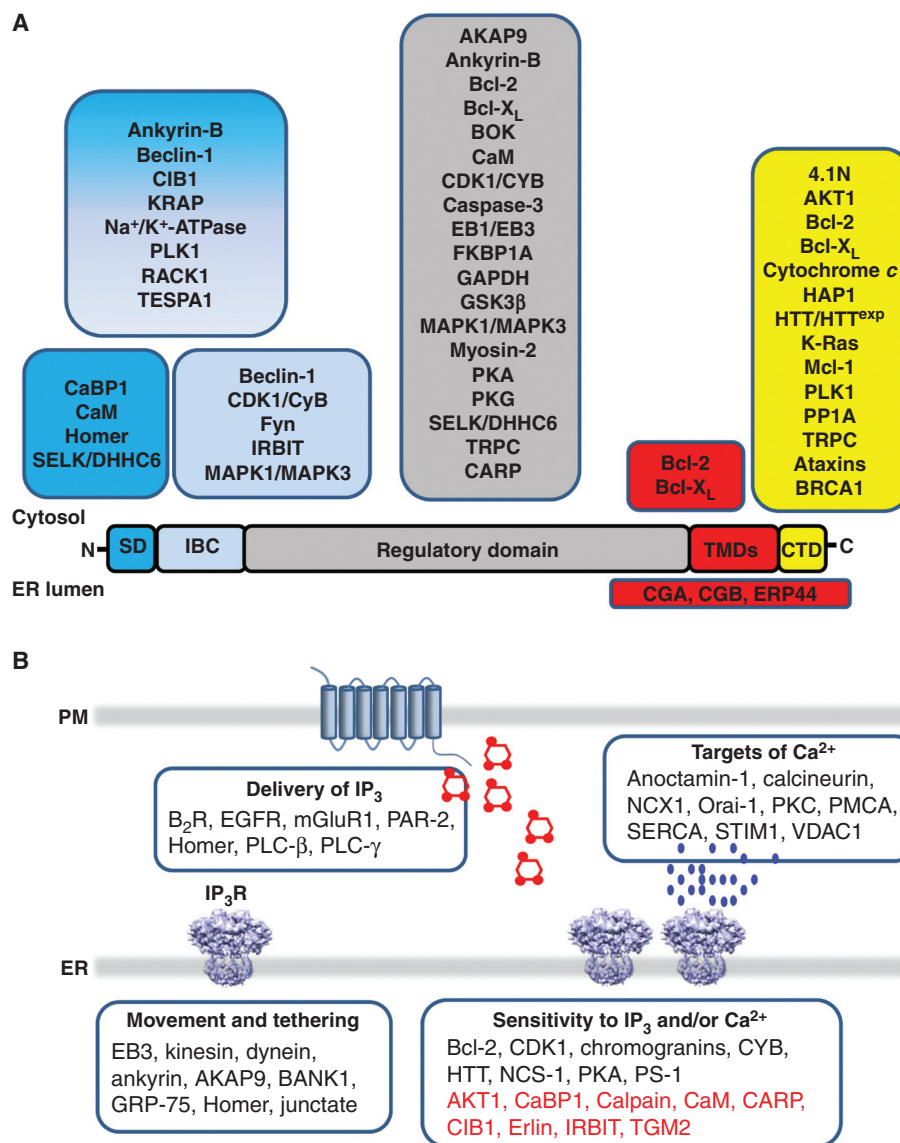


Figure 4. IP₃ receptors interact with many accessory proteins. (A) Proteins that interact with IP₃Rs shown according to the regions of the IP₃R with which they interact. (From Prole and Taylor 2016; adapted under the terms of the Creative Commons Attribution License [CC BY, 2016].) AKAP9, A-kinase anchoring protein 9; AKT1, RAC- α serine/threonine protein kinase; BANK1, B-cell scaffold protein with ankyrin repeats; Bcl-2, B-cell lymphoma 2; B₂R, bradykinin B₂ receptor; BRCA1, breast and ovarian cancer susceptibility gene 1; CaBP1, Ca²⁺-binding protein 1; CaM, calmodulin; CARP, carbonic anhydrase-related protein; CDK1, cyclin-dependent kinase 1; CIB1, Ca²⁺- and integrin-binding protein 1; CYB, cyclin-B1; EB3, end-binding protein 3; EGFR, epidermal growth factor receptor; GRP-75, glucose-regulated protein 75; HTT, huntingtin; IRBIT, IP₃-binding protein released with IP₃; mGluR1, metabotropic glutamate receptor 1; NCS-1, neuronal Ca²⁺-sensor 1; PAR-2, protease-activated receptor 2; PKA, protein kinase A; PLC- β , phospholipase C β ; PLC- γ , phospholipase C γ ; PS-1/PS-2, presenilin 1/2; NCX1, Na⁺/Ca²⁺ exchanger 1; PKC, protein kinase C; PMCA, plasma membrane Ca²⁺-ATPase; SERCA, sarco/endoplasmic reticulum Ca²⁺-ATPase; STIM1, stromal interaction molecule 1; TGM2, transglutaminase-2; VDAC1, voltage-dependent anion channel 1. Proteins shown in red inhibit the activity of IP₃Rs. Original sources for additional interactions shown here are ataxins (Chen et al. 2008; Liu et al. 2009), CARP (Hirota et al. 2003), and BRCA1 (Hedgepeth et al. 2015). (B) Examples of proteins shown according to whether they facilitate delivery of IP₃ to IP₃Rs, intracellular distribution of IP₃Rs, IP₃R activation or delivery of Ca²⁺ to specific targets.

D.L. Prole and C.W. Taylor

tion that CaBP1 stimulates IP₃Rs has been challenged by three different groups (Haynes et al. 2004; Nadif Kasri et al. 2004; Li et al. 2013). There is a need to establish whether there are additional physiological means, other than through IP₃ and Ca²⁺, to stimulate IP₃R activation.

We need also to consider whether the scaffolding of signaling proteins by IP₃Rs serves only to funnel information toward an IP₃-evoked Ca²⁺ signal, or might these scaffolds fulfil additional, and possibly unrelated, roles. There is, for example, evidence that IP₃Rs, independent of their ability to release Ca²⁺ from the ER, can modulate SOCE (Chakraborty et al. 2016) and, since the mechanisms are not yet clear (Thillaiappan et al. 2019), they may arise through scaffolding of proteins by IP₃Rs.

Space limitations forbid comprehensive discussion of IP₃Rs and their accessory proteins (additional examples can be found in Prole and Taylor 2016). Instead, we show some of the proteins that intercede at different levels, from facilitating delivery of IP₃ to IP₃Rs to guiding presentation of their Ca²⁺ signals to specific targets (Fig. 4B). A clear theme is that assembly of signaling proteins around IP₃Rs provides many opportunities for local integration and processing of information, before it is returned to the cell as a Ca²⁺ signal.

LICENSING IP₃ RECEPTORS TO RESPOND

High-resolution optical microscopy with fluorescent Ca²⁺ indicators has revealed the subcellular organization of the Ca²⁺ signals evoked by IP₃. Since these recordings have succeeded in observing the openings of single IP₃Rs in situ, they have been aptly named “optical patch-clamp” recording (Parker and Smith 2010). The results of these analyses show that low concentrations of IP₃ evoke short-lived openings of single IP₃Rs (“Ca²⁺ blips”). Greater concentrations of IP₃ evoke “Ca²⁺ puffs,” which typically last ~100 msec and report the coordinated opening of a few IP₃Rs within a small cluster. These are thought to arise when Ca²⁺ released by one IP₃R rapidly ignites the activity of its IP₃-bound neighbors through CICR (Fig. 2C; Smith and Parker 2009). Ca²⁺ puffs may be the building

blocks of all IP₃-evoked Ca²⁺ signals because all three IP₃R subtypes can generate Ca²⁺ puffs with broadly similar properties (Mataragka and Taylor 2018). As stimulus intensities increase further, Ca²⁺ diffusing from one Ca²⁺ puff can recruit the activity of a more distant site, generating a regenerative Ca²⁺ wave that spreads across the cell (Rooney et al. 1990; Bootman et al. 1997; Marchant et al. 1999). Further increases in stimulus intensity increase the frequency of the Ca²⁺ waves. These are manifest as Ca²⁺ spikes or oscillations at the whole-cell level, as first reported by Peter Cobbold (Woods et al. 1986). Hence, both the spatial and temporal organization of cytosolic Ca²⁺ signals changes with stimulus intensity, and this has important functional consequences (Thurley et al. 2014; Samanta and Parekh 2017). It is immediately apparent that both the coregulation of IP₃Rs by Ca²⁺ and IP₃, and the geographical relationships between IP₃Rs, are important determinants of how far Ca²⁺ signals progress through this hierarchy of Ca²⁺ release events (Fig. 2).

Considerable evidence suggests that most IP₃Rs are mobile within ER membranes (Ferrerri-Jacobia et al. 2005; Fukatsu et al. 2010; Pantazaka and Taylor 2011; Smith et al. 2014; Thillaiappan et al. 2017) and that IP₃ and/or Ca²⁺ can regulate clustering of IP₃Rs (Wilson et al. 1998; Iwai et al. 2005; Tateishi et al. 2005; Chalmers et al. 2006; Tojyo et al. 2008; Rahman and Taylor 2009; Pantazaka and Taylor 2011). There is also evidence that clustering may be required for cells to generate effective Ca²⁺ signals (Geyer et al. 2015). There is, however, a conundrum because, whereas most IP₃Rs are mobile, the Ca²⁺ puffs evoked by IP₃ repeatedly initiate at the same fixed sites within a cell (Thomas et al. 1998; Smith and Parker 2009; Smith et al. 2009b; Keebler and Taylor 2017; Mataragka and Taylor 2018). Why should a small fraction of the IP₃Rs in a cell assume complete responsibility for generating Ca²⁺ puffs?

We recently addressed this problem using HeLa cells in which gene editing was used to tag endogenous IP₃R1 with EGFP so that we could simultaneously observe the distribution of IP₃Rs and the Ca²⁺ signals they evoke (Thillaiappan et al. 2017). The results show that most

IP₃Rs are assembled into small clusters or puncta, and while the number of IP₃Rs within each cluster varies considerably, there is an average of ~8 IP₃Rs per cluster. Super-resolution imaging reveals that although IP₃Rs remain within their puncta, they are often too far apart (>100 nm) for the IP₃Rs to be held together by direct interactions between them (Fig. 5A,B). Instead, we suggest that a scaffold, which may be formed by proteins or lipids, corrals IP₃Rs into puncta (Fig. 5C). The scaffold has yet to be identified. Most IP₃R puncta, which are present in ER throughout the cell, are mobile (>70%). Most IP₃Rs move by diffusion in ER membranes, but a small fraction is moved directionally by microtubule motors. Others have

shown (Smith et al. 2009a; Ellefsen and Parker 2018), and we have confirmed (Keebler and Taylor 2017; Thillaiappan et al. 2017), that although IP₃Rs are present throughout the cell, almost all Ca²⁺ puffs arise from IP₃Rs within ER that lies close to the PM. This occurs whether IP₃ is delivered to the cell through endogenous receptor-activated signaling pathways, which might locally deliver IP₃ immediately beneath the PM, or by flash-photolysis of caged-IP₃, which uniformly delivers IP₃ throughout the cell. Hence, the propensity of near-PM IP₃Rs to respond does not arise from selective delivery of IP₃. Indeed, exactly the same Ca²⁺ release sites respond after photolysis of caged-IP₃ or stimulation of endogenous signaling pathways

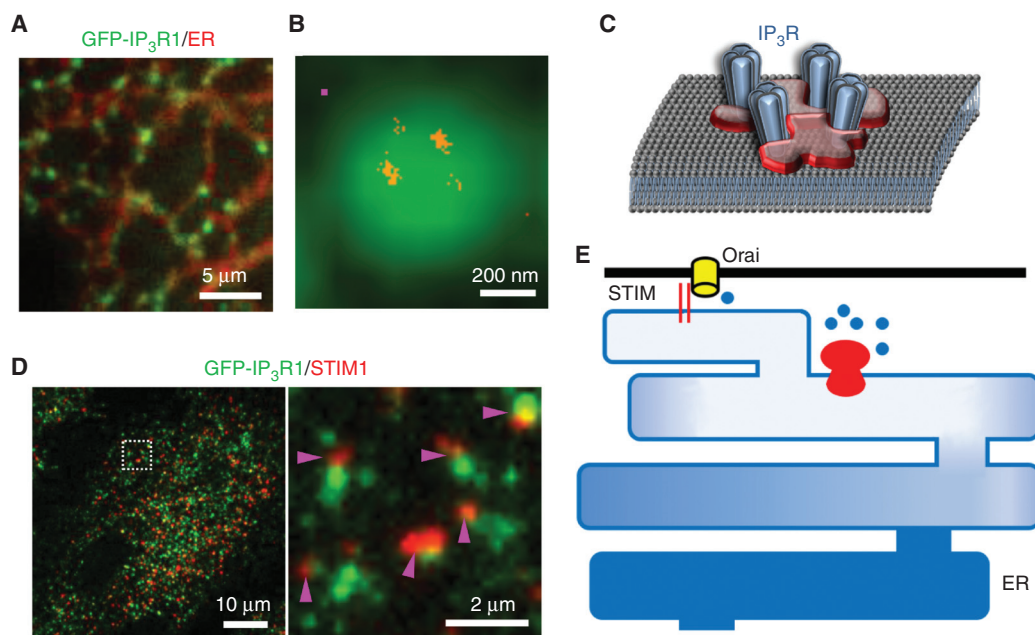


Figure 5. Immobile IP₃ receptor clusters initiate Ca²⁺ signals. (A) HeLa cells with endogenous IP₃R1 tagged with EGFP showing IP₃R puncta (green) within endoplasmic reticulum (ER) membranes (red). (B) Diffraction-limited image of a punctum recorded using total internal reflection fluorescence (TIRF) microscopy, and the superimposed super-resolution (STORM) image showing IP₃Rs (red) within the punctum. The magenta square indicates the approximate size of a single tetrameric IP₃R. (C) IP₃Rs appear to be diffusively distributed within a punctum, suggesting the need for a scaffold to corral IP₃Rs. (D) TIRF images of a HeLa cell in which the ER has been depleted of Ca²⁺ by treatment with thapsigargin, showing the distribution of STIM1 (red) and IP₃R (green). The enlarged image shows that stromal interaction molecule (STIM) puncta form alongside the ER where the immobile IP₃Rs that are licensed to respond are parked. (Data results for A–D are from Thillaiappan et al. 2017.) (E) Licensed IP₃Rs parked alongside the ER–PM junctions where store-operated Ca²⁺ entry (SOCE) occurs may allow substantial loss of Ca²⁺ from that part of the ER without causing appreciable Ca²⁺ loss from the remaining ER. The ER–PM junction with its associated licensed IP₃Rs may constitute the basic functional unit for SOCE.

D.L. Prole and C.W. Taylor

(Keebler and Taylor 2017; Lock et al. 2017). Furthermore, it is only the immobile IP₃R puncta adjacent to the PM that initiate Ca²⁺ puffs (Thillaiappan et al. 2017). It is, therefore, only a small fraction of the several thousand IP₃Rs expressed in a cell that are competent, or “licensed,” to respond. It will be important to identify the factor that licenses this small cohort of IP₃Rs to respond to IP₃.

We noted in a preceding section that IP₃R activation and SOCE are an almost universal partnership (Fig. 1B). It is, therefore, intriguing that the ER–PM junctions where STIM1 accumulates after loss of Ca²⁺ from the ER sit alongside the sites where licensed IP₃Rs reside (Fig. 5D; Thillaiappan et al. 2017). This too may have functional consequences by allowing active IP₃Rs to locally and substantially deplete ER specifically located alongside ER–PM junctions. This provides a possible answer to another problem, namely, that Ca²⁺ within the ER lumen has many roles beyond Ca²⁺ signaling—it is required for protein folding, for example—yet STIM1 is activated only after substantial loss of Ca²⁺ from the ER (Brandman et al. 2007; Luik et al. 2008; Bird et al. 2009). How does the ER regulate SOCE without compromising its other functions? We suggest that licensed IP₃Rs held alongside ER–PM junctions may be the basic functional units of SOCE, allowing IP₃ to cause a substantial loss of Ca²⁺ from the ER that regulates SOCE without trespassing into the remaining ER (Fig. 5E; Thillaiappan et al. 2017, 2019; Taylor and Machaca 2019).

CONCLUDING REMARKS

IP₃Rs allow Ca²⁺ to be redistributed, in response to receptor activation, from the ER lumen to the cytosol or other organelles, and they ultimately control SOCE. Coregulation of IP₃Rs by IP₃ and Ca²⁺ endows IP₃Rs with a capacity to mediate regenerative Ca²⁺ signals, which give rise to a hierarchy of Ca²⁺-release events as stimulus intensities increase. Recent progress with x-ray crystallography and cryo-EM have brought us closer to understanding how IP₃ binding to a site located some 7 nm from the pore, triggers Ca²⁺ binding and thence opening of the pore.

High-resolution measurements of Ca²⁺ signals in living cells have revealed that only a small cohort of immobile IP₃R clusters, parked alongside the ER–PM junctions where SOCE occurs, are licensed to respond. These IP₃Rs both mediate Ca²⁺ puffs and they may selectively regulate SOCE.

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D.L. Prole and C.W. Taylor



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D.L. Prole and C.W. Taylor

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