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Structure and Function of IP_3 Receptors

David L. Prole and Colin W. Taylor

Department of Pharmacology, University of Cambridge, Cambridge CB2 1PD, United Kingdom

Correspondence: cwt1000@cam.ac.uk

Inositol 1,4,5-trisphosphate receptors (IP_3Rs), by releasing Ca^{2+} from the endoplasmic reticulum (ER) of animal cells, allow Ca^{2+} to be redistributed from the ER to the cytosol or other organelles, and they initiate store-operated Ca^{2+} entry (SOCE). For all three IP_3R subtypes, binding of IP_3 primes them to bind Ca^{2+}, which then triggers channel opening. We are now close to understanding the structural basis of IP_3R activation. Ca^{2+}-induced Ca^{2+} release regulated by IP_3 allows IP_3Rs to regeneratively propagate Ca^{2+} signals. The smallest of these regenerative events is a Ca^{2+} puff, which arises from the nearly simultaneous opening of a small cluster of IP_3Rs. Ca^{2+} puffs are the basic building blocks for all IP_3-evoked Ca^{2+} signals, but only some IP_3 clusters, namely those parked alongside the ER–plasma membrane junctions where SOCE occurs, are licensed to respond. The location of these licensed IP_3Rs may allow them to selectively regulate SOCE.

Inositol 1,4,5-trisphosphate receptors (IP_3Rs) are expressed in most animal cells, including single-celled protozoa (Prole and Taylor 2011). They mediate release of Ca^{2+} 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). IP_3Rs are also expressed in the nuclear envelope and nucleoplasmic reticulum (Echevarría et al. 2003), where they may selectively generate nuclear Ca^{2+} signals, although cytosolic Ca^{2+} signals also invade the nucleoplasm (Bading 2013). IP_3R-mediated Ca^{2+} fluxes across ER membranes increase the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$), 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 Ca^{2+}. The accompanying decrease in ER luminal Ca^{2+} 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 Ca^{2+} channel in the PM (Hou et al. 2012; Yen and Lewis 2018), causing it to open (Prakriya and Lewis 2015). The resulting store-operated Ca^{2+} entry (SOCE) is almost universally associated with IP_3-evoked Ca^{2+} release. Hence, in response to the many extracellular stimuli that evoke IP_3 formation, IP_3Rs allow Ca^{2+} to be rapidly redistributed from the ER to the cytosol or other organelles and, by controlling the Ca^{2+} content of the ER, IP_3Rs control Ca^{2+} flowing into the cell through SOCE (Fig. 1).

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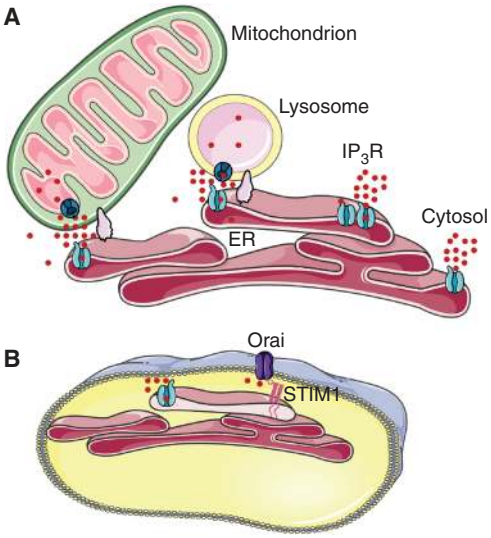

Figure 1. IP_3 receptors deliver Ca^{2+} to the cytosol and organelles. (*A*) By releasing Ca^{2+} from the endoplasmic reticulum (ER), IP_3Rs can deliver Ca^{2+} to the cytosol, to other IP_3Rs to ignite regenerative signals, or to the close appositions (membrane contact sites, supported by scaffold proteins) between the ER and other organelles. The latter include mitochondria and lysosomes, which can then accumulate Ca^{2+} via their low-affinity uptake systems from the high local Ca^{2+} concentration provided by IP_3Rs. (*B*) Loss of Ca^{2+} from the ER also activates STIM1, which then binds to Orai at ER–plasma membrane (PM) junctions to initiate store-operated Ca^{2+} entry (SOCE).

The cytosolic Ca^{2+} signals evoked by IP_3Rs 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 IP_3Rs to deliver Ca^{2+} to the mitochondrial uniporter (MCU) allows regulation of oxidative phosphorylation (Cardenas et al. 2010, 2016) and apoptosis (La Rovere et al. 2016), and Ca^{2+} delivery to lysosomes may allow them to accumulate Ca^{2+}, which regulates their activities (Xu and Ren 2015). Dysregulation of IP_3Rs 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 IP_3Rs in both normal physiology and disease, the only known antagonists of IP_3Rs (heparin, caffeine, and Xestospongin) lack specificity (Saleem et al. 2014). There is a pressing need for selective, membrane-permeant IP_3R antagonists.

Ca^{2+}-binding sites, which include those on the proteins that decode Ca^{2+} signals, are so abundant in cytosol that only some 1% of Ca^{2+} entering the cytosol remains free, although that buffering capacity does vary widely between cell types (Schwaller 2012). A cytosolic Ca^{2+} 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 Ca^{2+} diffuses slowly (Allbritton et al. 1992). This is an important feature that allows Ca^{2+} to linger at open channels and underpins its ability to serve as a local messenger. Hitherto, it has been assumed that IP_3 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 Ca^{2+} is a local messenger, while IP_3 is a global messenger. However, IP_3Rs in *Xenopus* ooctyes 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 IP_3Rs considered alongside their affinity for IP_3 and the necessity for an IP_3R to bind four molecules of IP_3 before it can open (Alzayady et al. 2016) suggest that IP_3Rs may, and prior to their activation, appreciably buffer IP_3 (Taylor and Konieczny 2016). Estimates of IP_3 diffusion in SH-SY5Y neuroblastoma cells, derived from measuring the extent to which IP_3 focally released from a caged precursor spreads to initiate local Ca^{2+} signals, have elegantly confirmed that diffusion of IP_3 in cells (diffusion coefficient, $D \sim 10\ \mu m^2/sec$) is ~30-fold slower than expected (Dickinson et al. 2016) and comparable to Ca^{2+} diffusion ($D = 13–65\ \mu m^2/sec$) (Allbritton et al. 1992). This suggests that both intracellular messengers,

IP_3 and Ca^{2+}, can act locally within the confines of a typical cell (Dickinson et al. 2016). The activities of many cells are coordinated by Ca^{2+} waves that spread between cells (Leybaert and Sanderson 2012). Diffusion of IP_3 through intercellular gap junctions is one means by which such Ca^{2+} waves are thought to propagate, but that idea was influenced by the assumption that IP_3 diffusion is unhindered (Leybaert 2016). The discovery that IP_3 diffuses slowly may require reappraisal of current thinking on how intercellular Ca^{2+} waves propagate and it invites speculation that there may be "highways" between cells wherein IP_3 buffering is reduced to facilitate faster intercellular diffusion.

In a contribution to the first edition of this collection, we reviewed the history of IP_3Rs (Taylor and Tovey 2012), noting that it was entwined with that of ryanodine receptors (RyRs), the close cousins of IP_3Rs. The cross fertilization between studies of these two major families of intracellular Ca^{2+} 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 IP_3R activation, evidence that IP_3Rs are regulated by many additional proteins, and the organization of IP_3Rs 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 IP_3Rs 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 IP_3Rs (Alzayady et al. 2015), and relationships between SOCE and IP_3Rs (Taylor and Machaca 2019; Thillaiappan et al. 2019). We begin with a short overview of IP_3Rs.

IP_3 RECEPTORS ARE REGULATED BY IP_3 AND Ca^{2+}

Vertebrate genomes encode subunits for three closely related IP_3R subunits ($IP_3R1–3$), which assemble into homo- and heterotetrameric channels. The subunits are enormous (~2700 residues), such that IP_3Rs and RyRs (which are even larger, $4 \times \sim 5000$ residues/RyR) are the largest known ion channels. The IP_3R 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 IP_3 ($IP_3R2 > IP_3R1 > IP_3R3$) (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 Ca^{2+} signals (Miyakawa et al. 1999; Wang and Yule 2018), and the functional consequences of perturbing IP_3Rs differ for the different subtypes (Hisatsune and Mikoshiba 2017). Despite the differences, the core functional properties of all IP_3Rs 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 IP_3Rs form large-conductance cation channels with relatively weak selectivity for Ca^{2+} over K^+ ($P_{Ca}/P_K \sim 7$) (Foskett et al. 2007). The large conductance, which allows a single IP_3R to conduct $\sim 10^5$ Ca^{2+}/sec or 1000 Ca^{2+} ions for each 10-msec opening (Vais et al. 2010), is important because it permits small numbers of IP_3Rs to rapidly deliver large local Ca^{2+} signals to the cytosol. The second feature common to all IP_3Rs, although historically it has spawned some controversy, is their biphasic regulation by $[Ca^{2+}]_c$. The activity of all IP_3Rs is enhanced by modest increases in $[Ca^{2+}]_c$ and inhibited by more substantial increases (Iino 1990; Bezprozvanny et al. 1991; Foskett et al. 2007). Whether IP_3Rs are also regulated directly by Ca^{2+} within the ER lumen remains a contentious and unresolved issue (Irvine 1990; Nunn and Taylor 1992; Vais et al. 2012).

Activation of IP_3Rs requires binding of both IP_3 to all four of its subunits (Alzayady et al. 2016) and Ca^{2+} binding (Finch et al. 1991; Marchant and Taylor 1997). The simplest scheme envisages two Ca^{2+}-binding sites associated with the IP_3R (Marshall and Taylor 1994). Biophysical analyses tentatively suggest that the stimulatory Ca^{2+}-binding site may be closer to

the pore than the inhibitory site (Vais et al. 2012). Different schemes have been proposed to explain the interaction between IP_3 and Ca^{2+}, with IP_3 proposed to regulate only the inhibitory Ca^{2+}-binding site (reducing its affinity for Ca^{2+}) (Mak et al. 1998; Vais et al. 2012) or both the inhibitory (reducing its Ca^{2+} affinity) and stimulatory (increasing its Ca^{2+} affinity) sites (Marchant and Taylor 1997; Adkins and Taylor 1999). Whatever the detailed mechanism, the outcome is that IP_3 primes IP_3Rs to respond to stimulation by Ca^{2+}, by either divorcing the stimulatory and inhibitory effects, or by directly promoting Ca^{2+} binding to the stimulatory site (Fig. 2A,B). This interplay has important implications because it allows IP_3Rs, in the presence of IP_3, to propagate Ca^{2+} signals regeneratively by Ca^{2+}-induced Ca^{2+} release (CICR) (Fig. 2C). We return to this feature later, but first we consider progress toward understanding the structural basis of how IP_3 and Ca^{2+} binding together lead to opening of a large-conductance channel through which Ca^{2+} can leave the ER.

HOW TO OPEN AN IP_3 RECEPTOR

How does IP_3 binding to a site, the IP_3-binding core (IBC), located ~7 nm from the constriction within the closed channel, lead to channel opening? Progress toward answering this question has come from high-resolution crystal structures of the amino-terminal region of the IP_3R, 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 IP_3R. Cryoelectron microscopy (cryo-EM) structures of IP_3R1 (Fan et al. 2015, 2018) and of IP_3R3 with and without IP_3 and Ca^{2+} (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 IP_3Rs.

The structure of the IP_3R 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 IP_3Rs 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

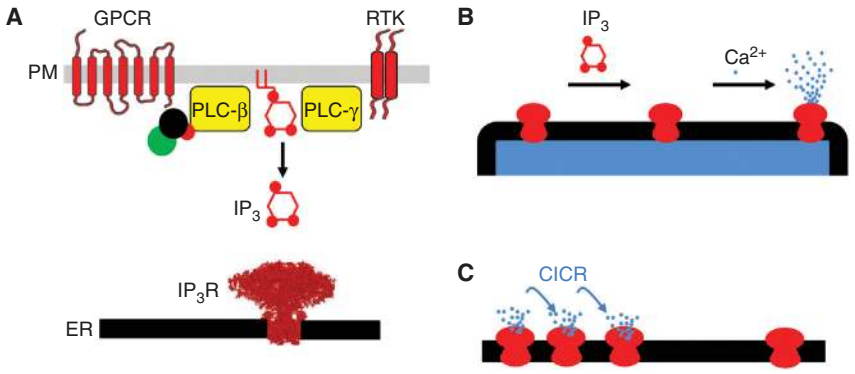

Figure 2. IP_3 receptors are stimulated by IP_3 and Ca^{2+}. (*A*) Many receptors, including G-protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs), can stimulate phospholipase C (PLC), leading to production of IP_3, which then binds to IP_3Rs in the endoplasmic reticulum (ER). (*B*) IP_3 binding to IP_3R primes them to bind Ca^{2+}, which then stimulates the channel to open, allowing Ca^{2+} to flow out of the ER. (*C*) This dual regulation of IP_3Rs by IP_3 and Ca^{2+} allows them to mediate regenerative signals propagated by Ca^{2+}-induced Ca^{2+} release (CICR). PM, Plasma membrane.

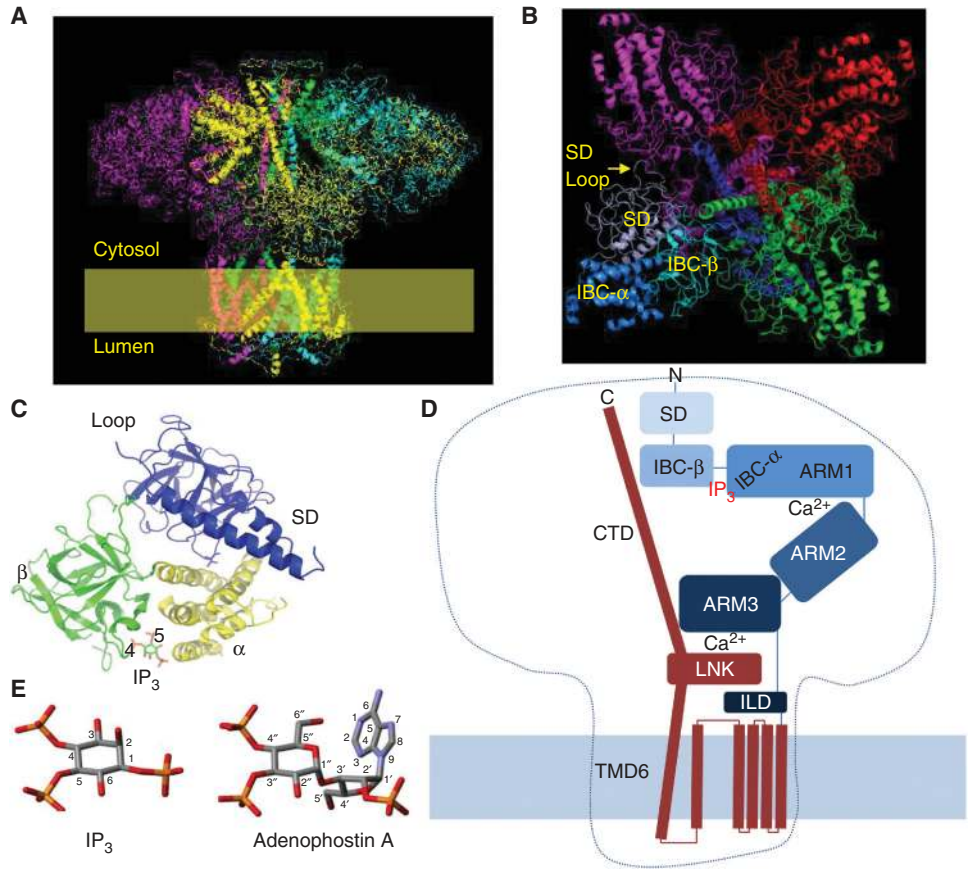

Figure 3. IP_3 receptor structure. (*A*) Cryoelectron microscopy (cryo-EM) of IP_3R1 shows its tetrameric mushroom-like structure. (From Fan et al. 2015; adapted, with permission, from Springer Nature © 2015.) Subunits are color-coded. A similar structure has been reported for IP_3R3 (Paknejad and Hite 2018). (*B*) View from the cytosol. (*C*) The amino-terminal region of each IP_3R subunit comprises the suppressor domain (SD) with the "hot spot" loop through which it contacts an adjacent subunit; and the IP_3-binding core (IBC), with its α and β domains. The essential 4- and 5-phosphates of IP_3 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 IP_3R1 subunit, highlighting the IBC, where IP_3 binds, two Ca^{2+}-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 IP_3 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 IP_3 and adenophostin A, showing how the latter has structures equivalent to the essential 4- and 5-phosphates of IP_3.

for its cellular functions. IP_3Rs 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 IP_3Rs to accrete accessory proteins (see below) and it may allow them to more effectively deliver Ca^{2+} 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 IP_3R subunit, although a

recent report suggests the possible presence of two additional TMDs between TMD1 and TMD2 in IP_3Rs (Fan et al. 2018; Paknejad and Hite 2018) and perhaps also in RyR (des Georges et al. 2016). The structure of the IP_3R 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 (Phe^{2586} and Ile^{2590} in IP_3R1) must move for the pore to open, but there may also be changes around the pore helix to displace a positively charged residue (His^{2541}) 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 IP_3R 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 Zn^{2+}-finger motif and aligns parallel with the ER membrane (Fan et al. 2015; Paknejad and Hite 2018). The functional significance of the Zn^{2+}-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 IP_3R.

IP_3 binding to the clam-like IBC initiates IP_3R 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 IP_3 (Bosanac et al. 2002; Seo et al. 2012; Paknejad and Hite 2018). All four subunits must bind IP_3 before the channel can open (Alzayady et al. 2016). It is less clear whether all stimulatory Ca^{2+}-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 IP_3R. 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 IP_3R (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 IP_3 (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 IP_3 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 IP_3Rs have structures equivalent to the 4- and 5-phosphates of IP_3. The structures also demonstrate how endogenous dephosphorylation of IP_3 to 1,4-IP_2 effectively terminates Ca^{2+} 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 IP_3Rs than IP_3, and with structures, its 3″- and 4″-phosphates, equivalent to the 5- and 4-phosphates of IP_3, respectively (Fig. 3E; Rossi et al. 2010; but see Fan et al. 2018). Whereas loss of the 5-phosphate group from IP_3 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 IP_3 binding is linked to IP_3R 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 IP_3-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 IP_3R. Mutations within ILD disrupt IP_3R function (Hamada et al. 2017), and the LNK domain contributes a conserved residue to a Ca^{2+}-binding site at the base of the ARM3 domain (Fig. 3D; Paknejad and Hite 2018). This Ca^{2+}-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 IP_3Rs (des Georges et al. 2016). We note that in both RyR (Glu^{4032} in RyR1) (Du and MacLennan 1998) and IP_3R1 (Glu^{2100}) (Miyakawa et al. 2001), a conserved glutamate was proposed to contribute to the stimulatory Ca^{2+}-binding site. Indeed, and somewhat perplexingly, mutation of this residue affected both stimulation and inhibition of IP_3Rs by Ca^{2+} (Miyakawa et al. 2001). It is now clear from structural analyses that these conserved glutamates do not coordinate Ca^{2+} in either RyR (des Georges et al. 2016) or IP_3R (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 IP_3R, is absent from IP_3Rs (Fan et al. 2015), and nor does the EF-hand provide the essential Ca^{2+} regulation of RyRs (Guo et al. 2016). Hence, the conserved Ca^{2+}-binding site at the interface between the cytosolic (ARM3) and channel domains (LNK) suggests an appealing, but untested, link between Ca^{2+} and gating of the IP_3R, namely that IP_3 binding stabilizes this Ca^{2+}-binding site and Ca^{2+} binding to it then leads to opening of the pore. This proposal also aligns with the suggestion that the stimulatory Ca^{2+}-binding site may be close to the pore (Vais et al. 2012). The high-resolution structure of IP_3R3 recently identified another Ca^{2+}-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 Ca^{2+}-binding sites relates to stimulation and inhibition of IP_3Rs by cytosolic Ca^{2+}. It is, however, intriguing that both sites are formed by residues contributed by different domains, consistent with IP_3-evoked rigid-body domain movements influencing whether Ca^{2+} binds to the sites.

Recent progress has brought us close to seeing how IP_3 binding to the IBC causes pore residues some 7 nm away to move and allow Ca^{2+} to pass from the ER lumen to the cytosol (Fan et al. 2018; Paknejad and Hite 2018). IP_3 initiates IP_3R 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 IP_3 primes IP_3Rs to bind Ca^{2+}, which then triggers channel opening (Fig. 2B; Adkins and Taylor 1999), we speculate that an intervening step between IP_3 binding to the IBC and pore opening involves rearrangement of Ca^{2+}-binding sites at the ARM1–ARM2 interface or at the LNK–ARM3 nexus. The conformational changes evoked by Ca^{2+} 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).

IP_3 RECEPTORS AS SIGNALING HUBS

IP_3 and Ca^{2+} are the essential regulators of IP_3R gating (Fig. 2), but many intracellular signals,

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_3Rs 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_3Rs by caspase-3 (Hirota et al. 1999) or calpains (Magnusson et al. 1993) may contribute to their degradation. But after limited proteolysis, IP_3R fragments remain associated as a functional channel. Intriguingly, native and cleaved IP_3Rs respond differently, suggesting that proteolysis can provide more subtle regulation than merely down-regulating IP_3Rs (Alzayady et al. 2013; Wang et al. 2017).

More impressive still is the huge array of proteins that associate with IP_3Rs (Fig. 4A; Prole and Taylor 2016). These proteins, which may associate with cytosolic or luminal parts of the IP_3R, can regulate the distribution of IP_3Rs (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_3Rs, the sensitivity of Ca^{2+} release to IP_3 and Ca^{2+}, and they may allow IP_3Rs 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_3R subtype, cell type, and perhaps contingent on interactions between modulators (Ivanova et al. 2014; Prole and Taylor 2016). IP_3R-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_3Rs and human diseases, additional to those arising from loss or mutation of IP_3Rs (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_3R-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_3Rs 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_3Rs 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_3Rs 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_3Rs (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_3Rs directly, bypassing the need for IP_3. However, the sugges-

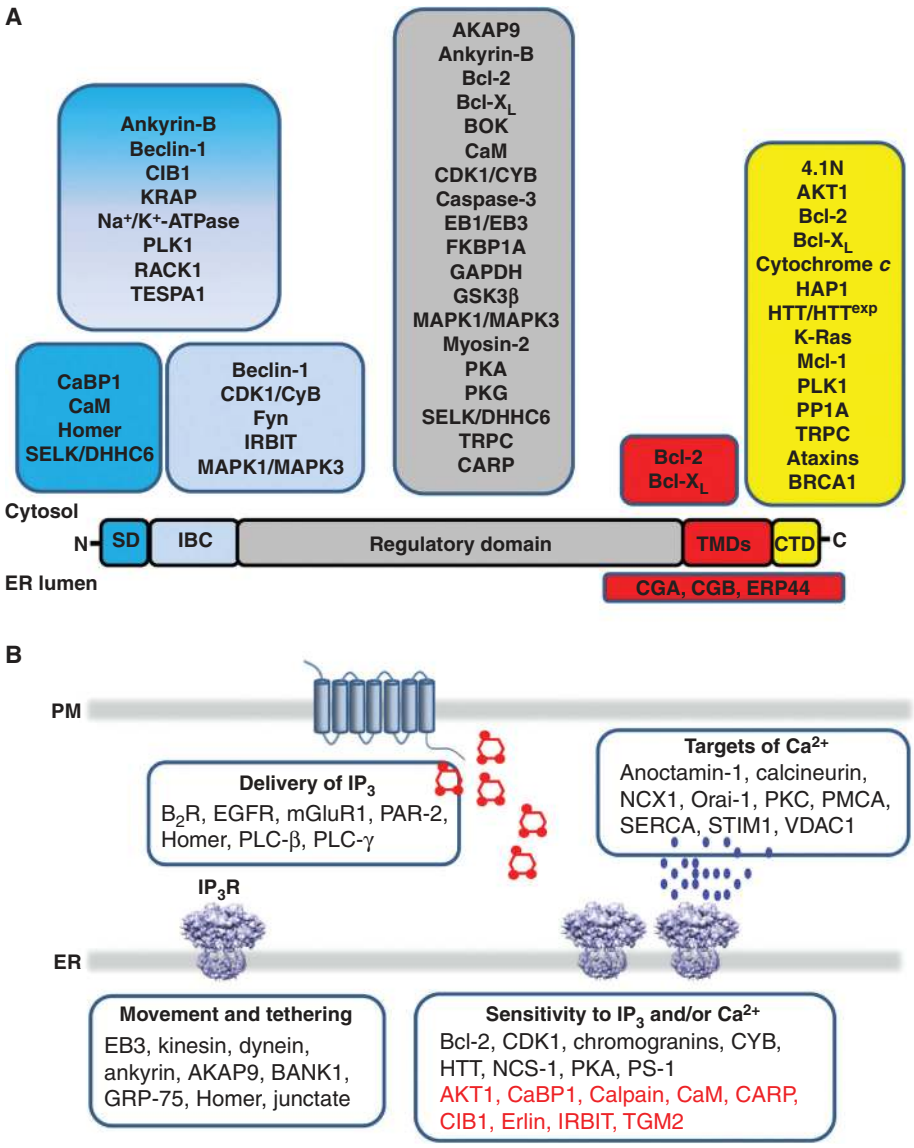

Figure 4. IP_3 receptors interact with many accessory proteins. (*A*) Proteins that interact with IP_3Rs shown according to the regions of the IP_3R with which they interact. (From Prole and Taylor 2016; adapted under the terms of the Creative Commons Attribution License [CC BY, 2016].) AKAP9, A-kinase anchoring protein 9; AKT1, RAC-α serine/threonine protein kinase; BANK1, B-cell scaffold protein with ankyrin repeats; Bcl-2, B-cell lymphoma 2; B_2R, bradykinin B_2 receptor; BRCA1, breast and ovarian cancer susceptibility gene 1; CaBP1, Ca^{2+}-binding protein 1; CaM, calmodulin; CARP, carbonic anhydrase-related protein; CDK1, cyclin-dependent kinase 1; CIB1, Ca^{2+}- 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, IP_3-binding protein released with IP_3; mGluR1, metabotropic glutamate receptor 1; NCS-1, neuronal Ca^{2+}-sensor 1; PAR-2, protease-activated receptor 2; PKA, protein kinase A; PLC-β, phospholipase Cβ; PLC-γ, phospholipase Cγ, PS-1/PS-2, presenilin 1/2; NCX1, Na^+/Ca^{2+} exchanger 1; PKC, protein kinase C; PMCA, plasma membrane Ca^{2+}-ATPase; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; STIM1, stromal interaction molecule 1; TGM2, transglutaminase-2; VDAC1, voltage-dependent anion channel 1. Proteins shown in red inhibit the activity of IP_3Rs. 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 IP_3 to IP_3Rs, intracellular distribution of IP_3Rs, IP_3R activation or delivery of Ca^{2+} to specific targets.

tion that CaBP1 stimulates IP_3Rs 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 IP_3 and Ca^{2+}, to stimulate IP_3R activation.

We need also to consider whether the scaffolding of signaling proteins by IP_3Rs serves only to funnel information toward an IP_3-evoked Ca^{2+} signal, or might these scaffolds fulfil additional, and possibly unrelated, roles. There is, for example, evidence that IP_3Rs, independent of their ability to release Ca^{2+} 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 IP_3Rs.

Space limitations forbid comprehensive discussion of IP_3Rs 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 IP_3 to IP_3Rs to guiding presentation of their Ca^{2+} signals to specific targets (Fig. 4B). A clear theme is that assembly of signaling proteins around IP_3Rs provides many opportunities for local integration and processing of information, before it is returned to the cell as a Ca^{2+} signal.

LICENSING IP_3 RECEPTORS TO RESPOND

High-resolution optical microscopy with fluorescent Ca^{2+} indicators has revealed the subcellular organization of the Ca^{2+} signals evoked by IP_3. Since these recordings have succeeded in observing the openings of single IP_3Rs 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 IP_3 evoke short-lived openings of single IP_3Rs ("Ca^{2+} blips"). Greater concentrations of IP_3 evoke "Ca^{2+} puffs," which typically last ~100 msec and report the coordinated opening of a few IP_3Rs within a small cluster. These are thought to arise when Ca^{2+} released by one IP_3R rapidly ignites the activity of its IP_3-bound neighbors through CICR (Fig. 2C; Smith and Parker 2009). Ca^{2+} puffs may be the building blocks of all IP_3-evoked Ca^{2+} signals because all three IP_3R subtypes can generate Ca^{2+} puffs with broadly similar properties (Mataragka and Taylor 2018). As stimulus intensities increase further, Ca^{2+} diffusing from one Ca^{2+} puff can recruit the activity of a more distant site, generating a regenerative Ca^{2+} 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 Ca^{2+} waves. These are manifest as Ca^{2+} 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 Ca^{2+} 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 IP_3Rs by Ca^{2+} and IP_3, and the geographical relationships between IP_3Rs, are important determinants of how far Ca^{2+} signals progress through this hierarchy of Ca^{2+} release events (Fig. 2).

Considerable evidence suggests that most IP_3Rs are mobile within ER membranes (Ferreri-Jacobia et al. 2005; Fukatsu et al. 2010; Pantazaka and Taylor 2011; Smith et al. 2014; Thillaiappan et al. 2017) and that IP_3 and/or Ca^{2+} can regulate clustering of IP_3Rs (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 Ca^{2+} signals (Geyer et al. 2015). There is, however, a conundrum because, whereas most IP_3Rs are mobile, the Ca^{2+} puffs evoked by IP_3 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 IP_3Rs in a cell assume complete responsibility for generating Ca^{2+} puffs?

We recently addressed this problem using HeLa cells in which gene editing was used to tag endogenous IP_3R1 with EGFP so that we could simultaneously observe the distribution of IP_3Rs and the Ca^{2+} signals they evoke (Thillaiappan et al. 2017). The results show that most

IP_3Rs are assembled into small clusters or puncta, and while the number of IP_3Rs within each cluster varies considerably, there is an average of ~8 IP_3Rs per cluster. Super-resolution imaging reveals that although IP_3Rs remain within their puncta, they are often too far apart (>100 nm) for the IP_3Rs 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 IP_3Rs into puncta (Fig. 5C). The scaffold has yet to be identified. Most IP_3R puncta, which are present in ER throughout the cell, are mobile (>70%). Most IP_3Rs move by diffusion in ER membranes, but a small faction 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 IP_3Rs are present throughout the cell, almost all Ca^{2+} puffs arise from IP_3Rs within ER that lies close to the PM. This occurs whether IP_3 is delivered to the cell through endogenous receptor-activated signaling pathways, which might locally deliver IP_3 immediately beneath the PM, or by flash-photolysis of caged-IP_3, which uniformly delivers IP_3 throughout the cell. Hence, the propensity of near-PM IP_3Rs to respond does not arise from selective delivery of IP_3. Indeed, exactly the same Ca^{2+} release sites respond after photolysis of caged-IP_3 or stimulation of endogenous signaling pathways

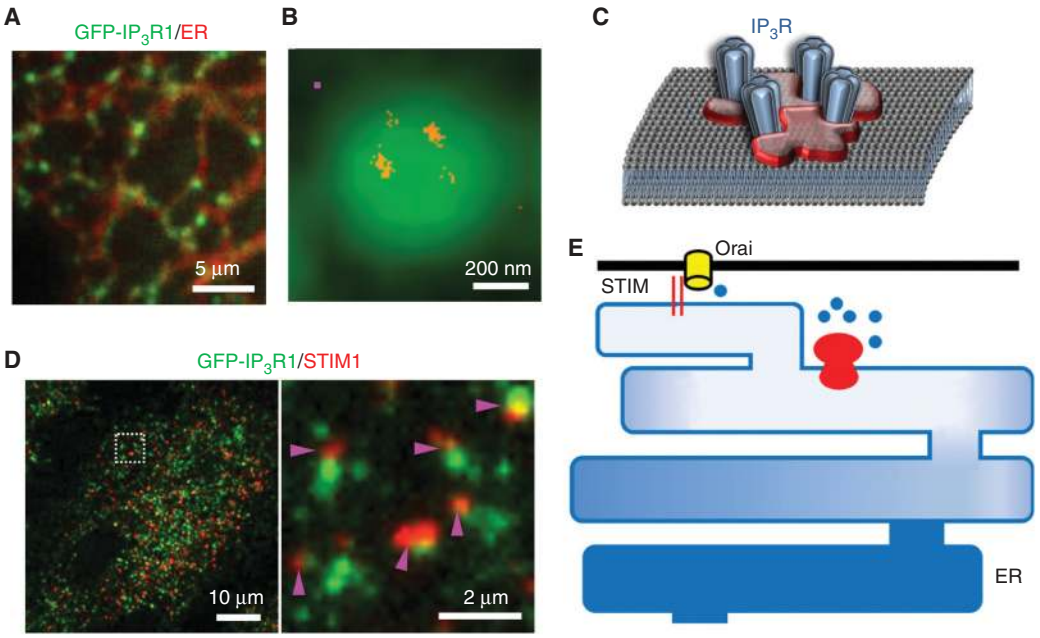

Figure 5. Immobile IP_3 receptor clusters initiate Ca^{2+}signals. (*A*) HeLa cells with endogenous IP_3R1 tagged with EGFP showing IP_3R puncta (green) within endoplasmic reticulum (ER) membranes (red). (*B*) Diffraction-limited image of a punctum recorded using total internal reflection fluorescence (TIRF) microscopy, and the superimposed super-resolution (STORM) image showing IP_3Rs (red) within the punctum. The magenta square indicates the approximate size of a single tetrameric IP_3R. (*C*) IP_3Rs appear to be diffusively distributed within a punctum, suggesting the need for a scaffold to corral IP_3Rs. (*D*) TIRF images of a HeLa cell in which the ER has been depleted of Ca^{2+} by treatment with thapsigargin, showing the distribution of STIM1 (red) and IP_3R (green). The enlarged image shows that stromal interaction molecule (STIM) puncta form alongside the ER where the immobile IP_3Rs that are licensed to respond are parked. (Data results for *A–D* are from Thillaiappan et al. 2017.) (*E*) Licensed IP_3Rs parked alongside the ER–PM junctions where store-operated Ca^{2+} entry (SOCE) occurs may allow substantial loss of Ca^{2+} from that part of the ER without causing appreciable Ca^{2+} loss from the remaining ER. The ER–PM junction with its associated licensed IP_3Rs may constitute the basic functional unit for SOCE.

(Keebler and Taylor 2017; Lock et al. 2017). Furthermore, it is only the immobile IP_3R puncta adjacent to the PM that initiate Ca^{2+} puffs (Thillaiappan et al. 2017). It is, therefore, only a small fraction of the several thousand IP_3Rs 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 IP_3Rs to respond to IP_3.

We noted in a preceding section that IP_3R 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 Ca^{2+} from the ER sit alongside the sites where licensed IP_3Rs reside (Fig. 5D; Thillaiappan et al. 2017). This too may have functional consequences by allowing active IP_3Rs to locally and substantially deplete ER specifically located alongside ER–PM junctions. This provides a possible answer to another problem, namely, that Ca^{2+} within the ER lumen has many roles beyond Ca^{2+} signaling—it is required for protein folding, for example—yet STIM1 is activated only after substantial loss of Ca^{2+} 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 IP_3Rs held alongside ER–PM junctions may be the basic functional units of SOCE, allowing IP_3 to cause a substantial loss of Ca^{2+} 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

IP_3Rs allow Ca^{2+} 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 IP_3Rs by IP_3 and Ca^{2+} endows IP_3Rs with a capacity to mediate regenerative Ca^{2+} signals, which give rise to a hierarchy of Ca^{2+}-release events as stimulus intensities increase. Recent progress with x-ray crystallography and cryo-EM have brought us closer to understanding how IP_3 binding to a site located some 7 nm from the pore, triggers Ca^{2+} binding and thence opening of the pore. High-resolution measurements of Ca^{2+} signals in living cells have revealed that only a small cohort of immobile IP_3R clusters, parked alongside the ER–PM junctions where SOCE occurs, are licensed to respond. These IP_3Rs both mediate Ca^{2+} puffs and they may selectively regulate SOCE.

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David L. Prole and Colin W. Taylor

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