IP_3 receptors and store-operated Ca^{2+} entry: a license to fill

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Inositol 1,4,5-trisphosphate receptors (IP_3Rs) are widely expressed intracellular Ca^{2+} channels that evoke large local increases in cytosolic Ca^{2+} concentration. By depleting the ER of Ca^{2+}, IP_3Rs also activate store-operated Ca^{2+} entry (SOCE). Immobile IP_3Rs close to the plasma membrane (PM) are the only IP_3Rs that respond to physiological stimuli. The association of these 'licensed' IP_3Rs with the ER-PM junctions where STIM interacts with Orai PM Ca^{2+} channels may define the autonomous functional unit for SOCE. Ca^{2+} entering cells through SOCE can be delivered directly to specific effectors, or it may reach them only after the Ca^{2+} has been sequestered by the ER and then released through IP_3Rs. This 'tunnelling' of Ca^{2+} through the ER to IP_3Rs selectively delivers Ca^{2+} to different effectors.

Highlights

- Structural studies are revealing how IP_3 and Ca^{2+} together cause the IP_3R to open
- Clusters of immobile IP_3Rs adjacent to the plasma membrane are licensed to respond
- SOCE signals directly to effectors and through IP_3Rs, after Ca^{2+} tunnelling via ER
- Licensed IP_3Rs may contribute to both activation of SOCE and Ca^{2+} tunnelling

Introduction

Inositol 1,4,5-trisphosphate receptors (IP_3Rs) are widely expressed intracellular Ca^{2+} channels through which IP_3 evokes Ca^{2+} release from the endoplasmic reticulum (ER) [1]. All IP_3Rs are large tetrameric channels that open only when they bind both IP_3 and Ca^{2+} [2]. This dual regulation allows IP_3Rs to propagate Ca^{2+} signals regeneratively, with IP_3 and the spatial distribution of IP_3Rs setting the gain on the Ca^{2+}-induced Ca^{2+} release (CICR) mechanism (**Figure 1a,b**). The rapid flux of Ca^{2+} through IP_3Rs generates signals on both sides of the ER membrane. On the cytosolic side, large local increases in Ca^{2+} concentration around active IP_3Rs can both propagate regeneratively between IP_3Rs to give global Ca^{2+} signals and fuel Ca^{2+} uptake by closely apposed organelles, like mitochondria [3] or lysosomes [4]. IP_3-evoked Ca^{2+} release thereby regulates Ca^{2+}-sensitive proteins in both the cytosol and organelles. The decrease in ER luminal Ca^{2+} concentration activates STIM1, which then accumulates at ER-plasma membrane (PM) junctions, where its interaction with Orai channels stimulates store-operated Ca^{2+} entry (SOCE) [5•]. Ca^{2+} provided by SOCE can regulate local effectors or, after sequestration by the ER, it can be 'tunnelled' to deliver Ca^{2+} through IP_3Rs to different targets [6••]. Here, we review recent progress towards understanding how IP_3 and Ca^{2+} regulate IP_3Rs, the assembly of IP_3Rs into spatially organized signalling units, and the implications of this organization for SOCE.

Towards a structural understanding of IP_3R activation

Progress towards understanding how IP_3 and Ca^{2+} control opening of the Ca^{2+}-permeable pore of the IP_3R has come from structural analyses of cytosolic fragments of the IP_3R [7-10,11•], cryo-EM structures of complete IP_3R1 [12••] and IP_3R3 [13••], and from similar analyses of the closely related family of intracellular Ca^{2+} channels, ryanodine receptors [14-19]. IP_3R activation begins when IP_3 partially closes the clam-like IP_3-binding core (IBC) [7,10,13••] on each of the four subunits of an IP_3R [20•] and it culminates with hydrophobic residues near the cytosolic end of the pore, and some 7 nm from the IBC, re-orienting to open a path through which hydrated Ca^{2+} ions can pass. It is not yet clear how conformational changes pass from IBC to pore, but they must traverse a nexus immediately above the ER membrane, where the underside of the huge cytosolic region extends into a leaflet that interdigitates with another leaflet at the end of the pore-lining helix (**Figure 1c,d**) [11•]. Residues provided by different parts of this structure together form a Ca^{2+}-binding site [13••,18••]. This architecture suggests an activation scheme wherein IP_3 binding may be communicated from the IBC to the nexus to stabilize a Ca^{2+}-binding site. Binding of Ca^{2+} to

this site, part of which is formed by the C-terminal extension of the pore-lining helix, might then cause dilation of the pore to create a path for Ca^{2+} to move rapidly from the ER lumen to the cytosol [21]. However, the function of this Ca^{2+}-binding site is unproven, and an additional Ca^{2+}-binding site, which also straddles different domains, was recently identified in the IP_3R cap [13••] (**Figure 1d**).

Structural analyses also shed light on another key feature of IP_3Rs, namely their ability to conduct large Ca^{2+} fluxes. The narrow selectivity filter at the luminal end of the pore is shorter and wider than in related channels, and sufficient to allow passage of hydrated Ca^{2+} ions [12••,16]. The cation-selectivity of the pore is provided by abundant acidic residues at its luminal and cytosolic vestibules. These features allow Ca^{2+} to move quickly through the pore (~$5x10^5$ Ca^{2+}/s *in situ*) [22] and, because the weak cation-selectivity of the pore allows K^+ to move as counterions [23], without the charge accumulation that would otherwise terminate Ca^{2+} release. These features allow IP_3Rs to rapidly generate large, local cytosolic Ca^{2+} signals.

Ca^{2+} puffs – licensed to respond

Pioneering work from Ian Parker's lab, using confocal [24], total internal reflection fluorescence microscopy (TIRFM) [25], and most recently lattice light-sheet microscopy [26] revealed a hierarchy of Ca^{2+} release events as the IP_3 concentration increases (**Figure 1b**). The smallest events, probably reflecting the opening of single IP_3Rs, are 'Ca^{2+} blips' that typically last only a few milliseconds. Greater stimulus intensities evoke larger and more long-lasting (~100 ms) 'Ca^{2+} puffs'. These report the nearly simultaneous opening of a few clustered IP_3Rs, as Ca^{2+} released by one IP_3R rapidly recruits the activity of its IP_3-bound neighbours. Similar Ca^{2+} puffs are observed in cells expressing each of the three IP_3R subtypes, suggesting they are a conserved feature of IP_3-evoked Ca^{2+} signalling [27]. As stimulus intensities increase further, Ca^{2+} diffusing between puffs ignites a regenerative wave that invades the entire cell. These waves become more frequent with increasing stimulus intensity. An important point is that the nature of the Ca^{2+} signal, local or global, and so the Ca^{2+} sensors recruited, changes with stimulus intensity.

There are, however, some puzzling features of these elementary Ca^{2+} release events. First, most IP_3Rs appear to be mobile yet Ca^{2+} puffs repeatedly initiate at fixed sites [28,29, see discussion in 30••]. What is so special about the few IP_3Rs that repeatedly respond? Second,

since all four IP_3-binding sites of an IP_3R must be occupied for the channel to open [20•], how do enough IP_3Rs within a small cluster become fully occupied and capable of generating a Ca^{2+} puff [31]? How is IP_3 binding distributed across IP_3Rs and what are the functional consequences? Third, numerous studies suggest regulated assembly of IP_3Rs into clusters [32 and references therein], but others suggest that the clusters from which Ca^{2+} puffs initiate are pre-assembled [33]. Does regulated clustering of IP_3Rs contribute to IP_3-evoked Ca^{2+} signals?

Whether activated by endogenous signalling pathways, where IP_3 might be selectively delivered to IP_3Rs [34], or by photolysis of caged-IP_3, where IP_3 is uniformly delivered to the cytosol, Ca^{2+} puffs initiate at the same fixed sites [28,29]. In each case, the sites are all close to the PM [26,30••]. By tagging native IP_3R with EGFP, it was possible to observe the geography of IP_3Rs as they evoke Ca^{2+} signals (**Figure 1d**) [30••]. Most IP_3Rs are assembled into rather loose clusters (each typically including ~8 IP_3Rs), wherein some IP_3Rs are several 100 nm apart. These clusters are distributed throughout the cell and most are mobile. However, the only IP_3R clusters that initiate Ca^{2+} puffs are those that are parked near the PM, adjacent to the ER-PM junctions where SOCE occurs. These IP_3Rs, a small fraction of the several thousand expressed in a cell, are 'licensed' to respond to IP_3. Neither the licensing factor nor the scaffold that holds IP_3Rs within their loose clusters has been identified. We speculate, based on patch-clamp analyses suggesting that IP_3 promotes local clustering of IP_3Rs [32], that IP_3 may cause the loose confederations of IP_3Rs to huddle more closely and so increase the opportunity for CICR between them. A similar clustering of IP_3Rs has been proposed to underlie the increased sensitivity of IP_3Rs to IP_3 as oocytes mature in preparation for fertilization [35].

It is not clear whether licensing of IP_3Rs increases their affinity for IP_3 and so diverts IP_3 binding to a subset of IP_3Rs, or whether licensing acts downstream of IP_3 binding. The latter would entail that to achieve complete occupancy of at least a few of the IP_3Rs within a licensed cluster, most IP_3Rs in the cell would have some of their subunits bound to IP_3. We might then ask whether partial occupancy of an IP_3R has functional consequences, possibly driving local clustering, for example [31,32]. Furthermore, since the intracellular concentration of IP_3Rs (~7200 IP_3Rs/cell, ~100 nM of IP_3-binding sites) is similar to their affinity for IP_3 (K_D = 119 nM under physiological conditions [36]), IP_3Rs may provide appreciable buffering and thereby slow IP_3 diffusion [31]. That appears to be the case; indeed the intracellular buffering of IP_3 may be greater than can be explained by IP_3Rs alone [37•].

This has important implications because, with IP_3 diffusing some 30-times slower than expected, its range of action is reduced. Hence, IP_3, like Ca^{2+} [38], probably functions as a local intracellular messenger [37•].

IP_3Rs and SOCE: short-cuts and tunnels

Activation of SOCE requires substantial loss of Ca^{2+} from the ER [39-41]. Yet the ER forms a luminally continuous network [42,43], and during Ca^{2+} signalling it must support other Ca^{2+}-requiring activities, including protein folding [44]. Furthermore, the ER-PM junctions where SOCE occurs, perhaps 400 junctions in a HeLa cell [5•], are just 10-20 nm wide and involve no more than a few percent of the PM area [5•,45]. How, then, do physiological stimuli both activate SOCE without compromising ER functions and deliver Ca^{2+} signals beyond ER-PM junctions? In most analyses, SOCE is activated by using thapsigargin to inhibit the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPases (SERCA) responsible for ER Ca^{2+} uptake. However, under physiological conditions, IP_3Rs mediate the loss of ER Ca^{2+} that triggers SOCE, and SERCA remain active throughout. Both features are important.

Licensed IP_3Rs are immobilized alongside the ER-PM junctions where SOCE occurs [30••]. These IP_3Rs, with their large Ca^{2+} conductances [22], may provide a transient 'short-circuit' for Ca^{2+} that briefly drains the ER within the ER-PM junction, thereby causing the large drop in ER Ca^{2+} concentration needed to activate SOCE (**Figure 2a**). The active IP_3Rs will also drain Ca^{2+} from the more central ER, but that Ca^{2+} pool is much larger and, with Ca^{2+} diffusing rapidly within the ER lumen [46], its luminal Ca^{2+} concentration will be minimally affected. These ideas lead to the proposal that each ER-PM junction forms an autonomous SOCE unit, regulated by its associated licensed IP_3Rs. We envisage this scheme operating during the low stimulus intensities that evoke Ca^{2+} oscillations, which are sustained by SOCE but with no discernible decrease in the overall ER Ca^{2+} concentration [39]. The model would allow IP_3Rs to stimulate SOCE without trespassing into other ER functions. It also entails only local movements of STIM after store depletion, which might allow local activation of SOCE to be very rapid.

Several effectors, including activation of the transcription factor NFAT by calcineurin [47] and some adenylyl cyclases [48] are directly regulated by Ca^{2+} signals evoked by SOCE, but for others the route from SOCE to effector is through the ER [6••,39,49-51•, reviewed in 52]. Here, Ca^{2+} entering the cell through SOCE is sequestered by the ER and then released back to

the cytosol through IP_3Rs that may be remote from ER-PM junctions where SOCE occurs [52]. This 'tunnelling' of Ca^{2+} from SOCE through the ER is reminiscent of the original 'capacitative' model for SOCE [53]. Patrick Hogan has estimated that the handful of active Orai channels within each maximally activated ER-PM junction collectively deliver ~30,000 Ca^{2+}/s [5•], most of which will rapidly diffuse out of the junction. By contrast a single IP_3R in a replete ER can deliver 500,000 Ca^{2+}/s to the cytosol, while SERCA working at its maximal capacity can remove fewer than 40 Ca^{2+}/s [5•,54]. Hence, unless SERCA are very concentrated within ER-PM junctions and adequately provisioned with ATP therein, they are unlikely to have much impact on Ca^{2+} concentrations within an active junction, but SERCA can deliver Ca^{2+} to the ER lumen and so sustain Ca^{2+} tunnelling [6••,51•,55,56]. Recent studies suggest three different categories of effectors for which SOCE can be important (**Figure 2b**). Effectors in the first category, typified by calcineurin [47], are closely associated with the SOCE apparatus and directly regulated by Ca^{2+} signals in the ER-PM junction. The second category, typified by Ca^{2+}-activated K^+ and Cl^- channels in the PM, is regulated by SOCE, but only after the Ca^{2+} has been sequestered by the ER and then released through nearby IP_3Rs [6••,51•]. These may be the licensed IP_3Rs that sit alongside the SOCE machinery (**Figure 2a,b**). The third category, which includes the mitochondrial uptake system (MCU) in HeLa cells [51•], is activated by IP_3-evoked Ca^{2+} release from replete ER, but not during SOCE and Ca^{2+} tunnelling. These effectors are probably coupled to IP_3Rs deeper within the cell. These IP_3Rs are deprived of Ca^{2+} when licensed IP_3Rs respond and divert Ca^{2+} sequestered from the ER-PM junctions back to the cytosol before it can fill the deeper ER [51•].

Concluding remarks

Remarkable progress has brought us close to understanding the structural basis of IP_3R activation and of the interactions between STIM and Orai that mediate SOCE [57]. Licensed IP_3Rs adjacent to the ER-PM junctions where SOCE occurs may contribute to both local activation of SOCE and allow delivery of Ca^{2+} tunnelled through the ER to specific intracellular effectors.

Conflicts of interest

CWT declares that he has no conflicts of interest. KM is a co-founder of Valdia Health, but declares no conflict of interest with this work.

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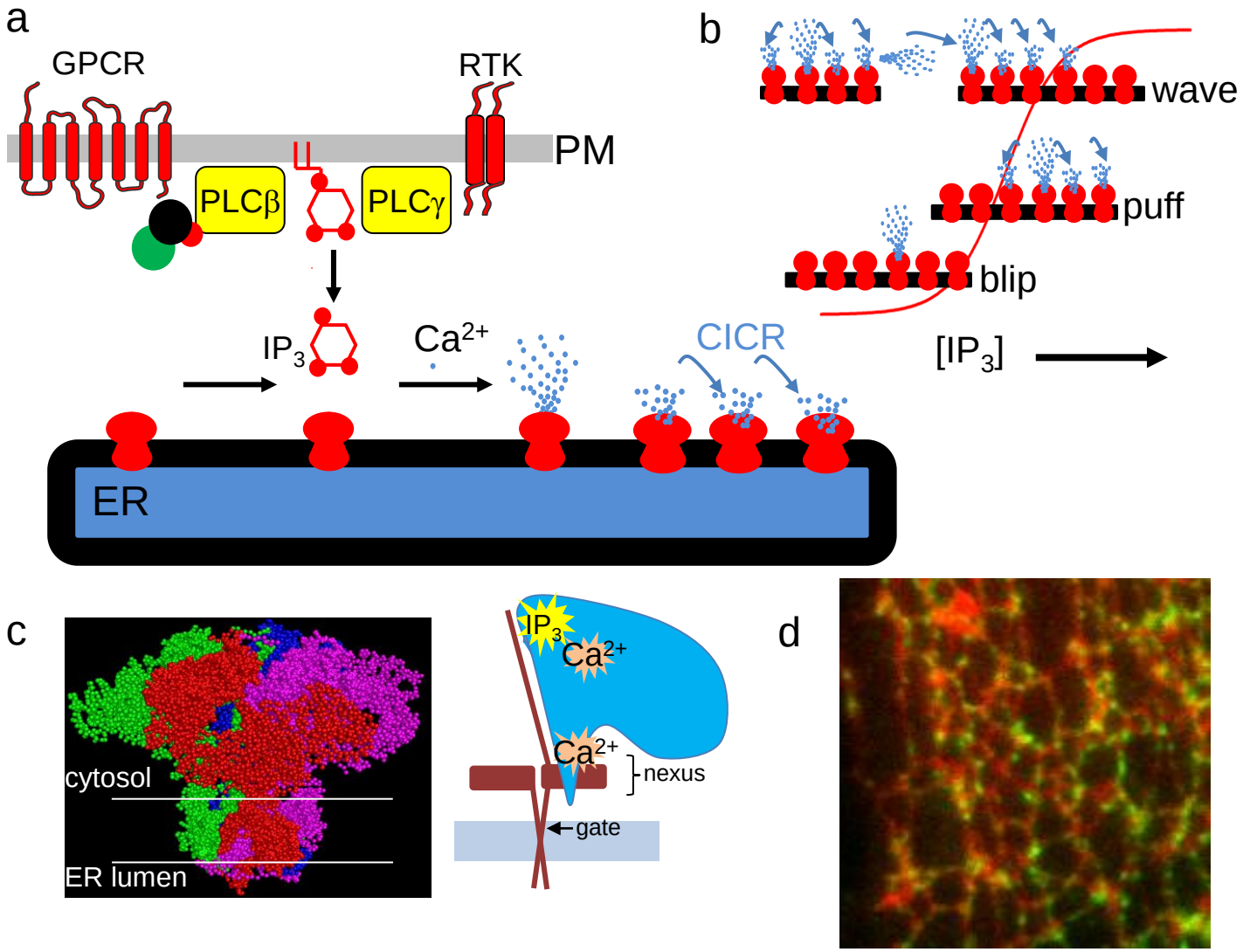
Figure 1

Regulation of IP_3Rs by IP_3 and Ca^{2+}. **(a)** IP_3 is produced when G-protein coupled receptors (GPCRs) or receptor tyrosine kinases (RTK) stimulate phospholipase C (PLC). IP_3 binding to IP_3Rs in the ER unmasks a Ca^{2+}-binding site, and when Ca^{2+} binds to it the channel can open. Ca^{2+} signals can then propagate regeneratively by Ca^{2+}-induced Ca^{2+} release (CICR). (**b**) As the IP_3 concentration increases, it evokes a hierarchy of Ca^{2+} release events. (**c**) The IP_3R is a large tetrameric mushroom-like structure [12••]. IP_3 binding near the cap of the mushroom causes the clam-like IBC to close and initiate conformation changes. These propagate to the pore-lining helices to cause the gate, formed by hydrophobic residues near the cytosolic surface of the ER membrane, to open. A nexus of entwined structures from the pore and cytosolic domains allow communication between them, and may provide a critical Ca^{2+}-binding site [13••,18••]. A second Ca^{2+}-binding site is formed at the interface of two of the cytosolic domains [13••]. **(d)** Within the ER (red), most IP_3Rs (green) assemble into small clusters of loosely associated IP_3Rs [30].

Figure 2

Licensed IP_3Rs and SOCE. (**a**) IP_3Rs licensed to respond to IP_3 are anchored alongside the ER-PM junctions where SOCE occurs. Activation of a licensed IP_3R cluster may 'short-circuit' the ER by transiently providing a large-conductance Ca^{2+} leak. The leak drains Ca^{2+} from the small ER region within the ER-PM junction, causing its luminal Ca^{2+} concentration to fall, providing an effective signal for activation of SOCE (right panel). Draining Ca^{2+} from the more extensive ER on the other side of the leak will minimally affect its luminal Ca^{2+} concentration. We suggest that the fundamental functional unit for SOCE is the ER-PM junction with its associated licensed IP_3Rs. (**b**) Ca^{2+} entering cells through SOCE can directly regulate effectors (1, eg, calcineurin) within ER-PM junctions. SERCA allows sequestration by the ER of some incoming Ca^{2+}, which may then be tunnelled through the ER lumen (red arrows) to licensed IP_3Rs close to the PM, from which it is released and regulates distinct effectors (2, eg, Ca^{2+}-activated K^+ channels). We suggest that the short-circuit provided by licensed IP_3Rs diverts Ca^{2+} to the cytosol before it can reach ER deeper within the cell. The IP_3Rs within this ER direct Ca^{2+} to different effectors only when the ER is replete with Ca^{2+} (3, eg, mitochondria in HeLa cells).


a
GPCR
RTK
PM
PLCβ
PLCγ
IP3
Ca2+
CICR
ER
b
wave
puff
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c
cytosol
ER lumen
IP3
Ca2+
Ca2+
nexus
gate
d

Figure 1

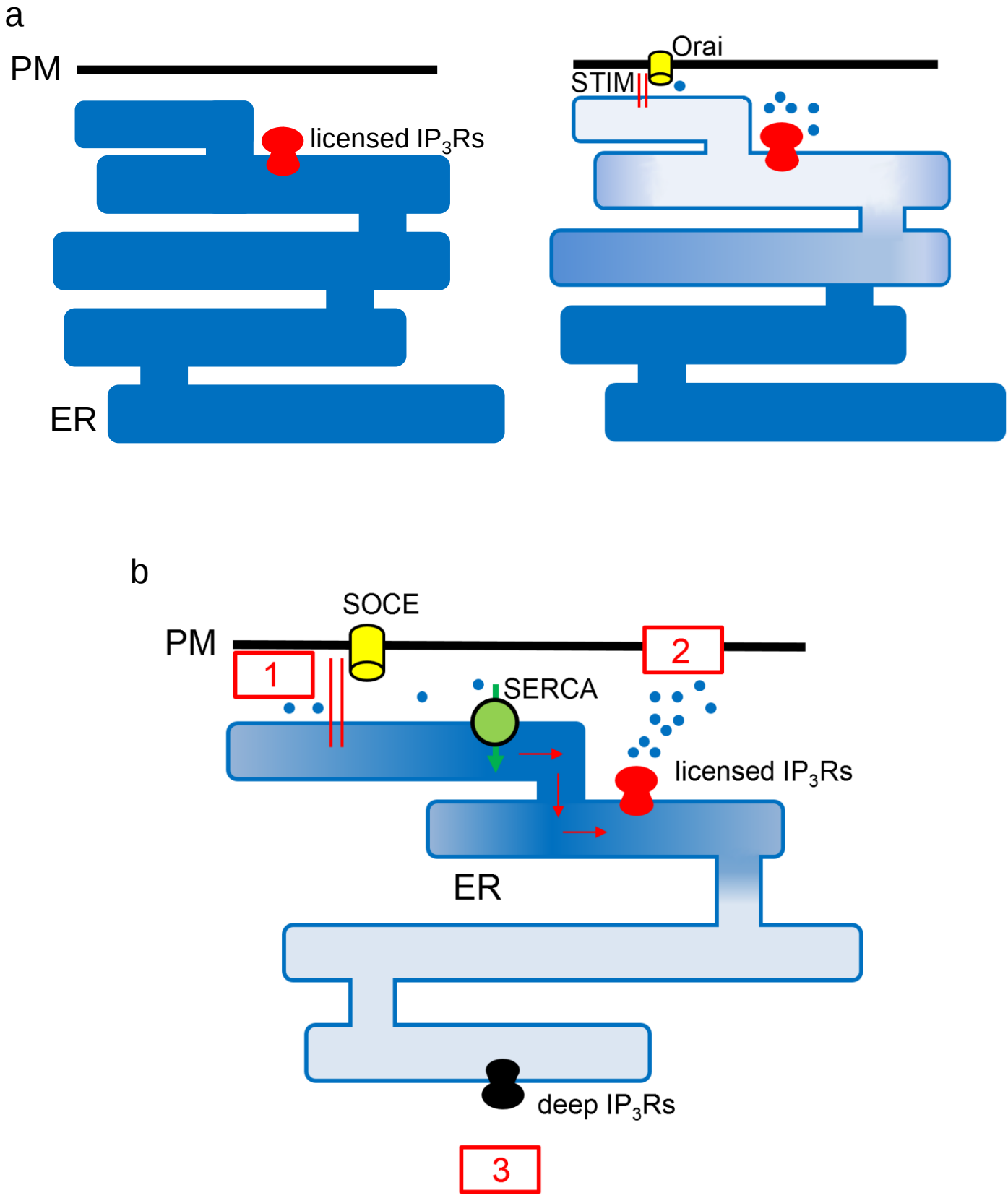
a
PM
licensed IP3Rs
ER
Orai
STIM
b
SOCE
PM
1
2
SERCA
licensed IP3Rs
ER
deep IP3Rs
3

Figure 2