

Localization and socialization: Experimental insights into the functional architecture of IP₃ receptors

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(Received 18 February 2009; accepted 11 May 2009; published online 18 September 2009)

Inositol 1,4,5-trisphosphate (IP₃)-evoked Ca²⁺ signals display great spatiotemporal malleability. This malleability depends on diversity in both the cellular organization and *in situ* functionality of IP₃ receptors (IP₃Rs) that regulate Ca²⁺ release from the endoplasmic reticulum (ER). Recent experimental data imply that these considerations are not independent, such that—as with other ion channels—the local organization of IP₃Rs impacts their functionality, and reciprocally IP₃R activity impacts their organization within native ER membranes. Here, we (i) review experimental data that lead to our understanding of the “functional architecture” of IP₃Rs within the ER, (ii) propose an updated terminology to span the organizational hierarchy of IP₃Rs observed in intact cells, and (iii) speculate on the physiological significance of IP₃R socialization in Ca²⁺ dynamics, and consequently the emerging need for modeling studies to move beyond gridded, planar, and static simulations of IP₃R clustering even over short experimental timescales. © 2009 American Institute of Physics. [DOI: [10.1063/1.3147425](https://doi.org/10.1063/1.3147425)]

Changes in cytoplasmic Ca²⁺ concentration control the activity of numerous cellular proteins. Inositol 1,4,5 trisphosphate receptors (IP₃Rs) localized on the endoplasmic reticulum play a key role in regulating cellular Ca²⁺ homeostasis by controlling Ca²⁺ flux into the cytoplasm from this intracellular Ca²⁺ store. IP₃R stimulation results in complex spatial and temporal patterns of intracellular Ca²⁺ release activity. Consequently, the utility of computational approaches in recapitulating and predicting Ca²⁺ release dynamics requires accurate definition of the subcellular dynamics and *in situ* functionality of cellular IP₃Rs. This review summarizes experimental data underpinning our current understanding of the functional architecture of IP₃Rs in the endoplasmic reticulum, to highlight the need for the next generation of modeling studies to consider the functional ramifications of IP₃R socialization within diverse and dynamic architectures in live cells.

I. INTRODUCTION

The cellular architecture of IP₃Rs is diverse: Their global distribution is not uniform, and their localized organization is not stereotypic. This organizational diversity is significant for patterning Ca²⁺ transients, crudely by dictating the targeting and propagation of Ca²⁺ signals (“localization”), but likely also by impacting the functional properties of IP₃Rs within the native membranes where they reside (“socialization”). Therefore, from an experimentalist’s perspective, predictive modelling of cellular Ca²⁺ signals necessitates dis-

secting the “functional architecture” of IP₃Rs,¹ to reflect both the cellular organization and unitary *in situ* properties of these channels, as well as the dynamic interdependence of these two considerations. This brief review will summarize recent experimental data on this theme, addressing the cellular organization of IP₃Rs from the macroscopic (cellular, “global”) down to the microscopic (subcellular, “local”) scale, ensuing ramifications for IP₃R function, and emerging modeling attempts to predict the dynamics and properties of IP₃Rs in different states of organization within realistic cellular geometries.

II. THE GLOBAL DISTRIBUTION AND LOCAL ORGANIZATION OF IP₃RS IS INHOMOGENOUS

There can be considerable macroscopic heterogeneity in IP₃R localization throughout the cell, both within and beyond the bulk endoplasmic reticulum (ER) (see Ref. 2 for a review). This is best exemplified in the gradients of IP₃R localization in polarized cells demonstrated by antibody/fluorescent ligand labeling, fractionation, and functional Ca²⁺ imaging approaches, as well by resolution in electron micrographs of variable IP₃R densities between different subcellular regions of ER.^{3–9} Similarly, targeting of IP₃R outside the “bulk” endoplasmic reticulum—at the cell surface,¹⁰ in other organelles,^{11,12} or within deep nuclear projections¹³—may further bias IP₃R distribution across the cell. Such gradients in endogenous IP₃R localization are crucial for cellular physiology by impacting resting Ca²⁺ gradients, as well as the initiation and the spatiotemporal dynamics of agonist-evoked Ca²⁺ signals. Often these differential distributions derive from differential targeting of IP₃R isoforms,^{2,6,7,14,15} but regionalization also occurs in cells where a single IP₃R iso-

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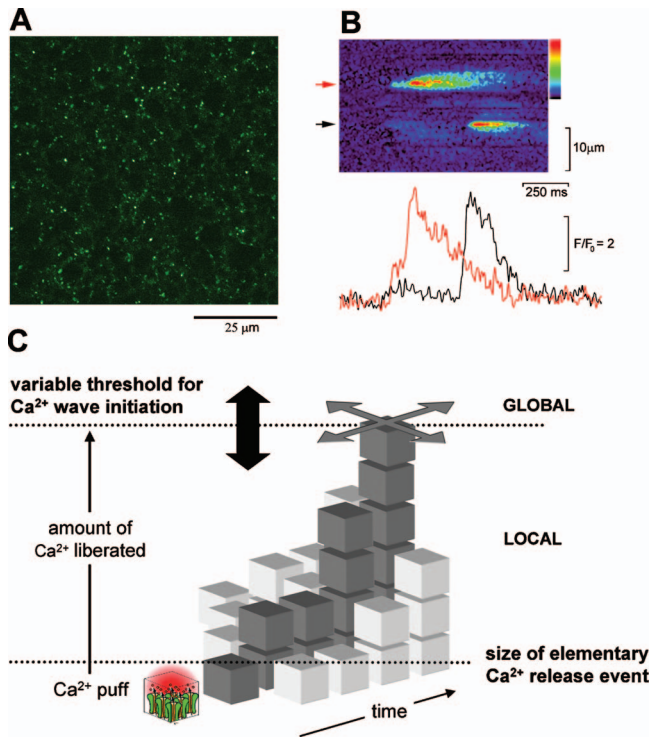


FIG. 1. (Color) Structural, functional, and conceptual views of native IP₃R architecture in the *Xenopus* oocyte. (a) Punctate immunolocalization of native clusters of IP₃Rs in the cortical ER of a *Xenopus* oocyte resolved by immunofluorescence. (b) Top: linescan image of two discrete Ca²⁺ puffs resolved using fluo-4 (K_d for Ca²⁺ = ~345 nM) that were evoked by photorelease of IP₃. Bottom: associated fluorescence profiles. (c) Schematic model, combining (a) and (b), conceptualizing Ca²⁺ puffs as the local elementary Ca²⁺ release occurring from discrete IP₃R clusters (cubes) spaced throughout the ER. Summation of the stochastic behavior of these elementary events is required to trigger a Ca²⁺ wave and establish repetitive Ca²⁺ oscillations. Ca²⁺ puffs occur independently at low levels of stimulation, but activity is coordinated by Ca²⁺ during maintained stimulation (x -axis) to trigger a global whole-cell Ca²⁺ signal. This summation is often coordinated by the activity of particular “focal” Ca²⁺ puffs sites (dark gray) that serve as the spatial focus of wave initiation. In the *Xenopus* oocyte, the amount of Ca²⁺ liberated by a Ca²⁺ puff is well below the threshold to initiate a Ca²⁺ wave, allowing the coexistence of local and global signaling modalities in the same cell.

form predominates (for example, Refs. 1, 9, and 16–18). Indeed, there is growing molecular insight into mechanisms dictating IP₃R localization in diverse cell types.^{2,19,20} While, obviously, graded spatial expression is but one way to vary intracellular Ca²⁺ release activity, it provides a simple route to customize cellular Ca²⁺ signals.

Similarly, at the microscopic level, the local organization of IP₃Rs within the ER is not homogenous. First, in peripheral ER closely juxtaposed to the cell surface, IP₃Rs frequently show punctate distributions within signaling complexes, localized by molecular interactions unique at this interface. The existence of such IP₃R-enriched “microdomains” identified in diverse cell types, serve to define agonist specificity and/or initiation sites of Ca²⁺ signals.^{21–28} Within nonperipheral ER, both structural and functional data suggest that IP₃Rs are gregarious, organizing into discrete “clusters” in “unstimulated” cells. For example, antibody staining reveals punctate IP₃R immunoreactivity throughout the ER in primary [Fig. 1(a)] (Refs. 16 and 29–31) and im-

mortalized cells,^{32,33} a conclusion also supported from a more limited pool of electron microscopy-based analyses.⁹ While careful collection and processing of immunocytochemical datasets can provide quantitative insight into the number, three dimensional distribution and properties of endogenous Ca²⁺ channel clusters,^{34,35} such methods have not yet been applied to IP₃Rs. Consequently, the majority of evidence supporting a native, clustered organization of IP₃Rs derives from functional rather than structural measurements, specifically electrophysiological analyses of endogenous IP₃R distributions in excised nuclei^{36,37} and predominantly from confocal Ca²⁺ imaging in intact cells.^{38,39}

Rapid, confocal Ca²⁺ imaging methods revealed that IP₃-evoked Ca²⁺ release from the ER was a discontinuous process, by demonstrating discrete and recurrent sites of Ca²⁺ release spaced throughout the ER. At low levels of stimulation, localized Ca²⁺ release events occurred at these sites spreading for a only few micrometers and persisting for a few hundred milliseconds [Fig. 1(b)]. These rapid, transient Ca²⁺ release events were christened Ca²⁺ “puffs”⁴⁰ and combined experimental and modeling analyses of Ca²⁺ puff properties have established that they result from the coordinated opening of a small number of Ca²⁺ channels.^{41,42} Similar “elementary” responses mediated by clusters of ryanodine receptors, the other major class of intracellular Ca²⁺ channels are discussed elsewhere.^{43,44} The variabilities in the amplitude of successive Ca²⁺ puff events and peak amplitude of Ca²⁺ puffs resolved at the same site suggest that not all IP₃Rs open during a Ca²⁺ puff and that the underlying number of clustered receptors varies between Ca²⁺ release sites.^{41,42} A recent experimental estimate, calibrating probable single channel responses (“Ca²⁺ blips”) to subsequent Ca²⁺ puffs that they rapidly trigger suggest between ~6 and 60 IP₃Rs open during a Ca²⁺ puff.⁴⁵ Deterministic simulations of the fluorescence profiles of these coupled events further refine these experimental estimates to suggest that Ca²⁺ puffs could result from the activity of ~25–35 IP₃Rs that simultaneously open for ~20 ms.⁴⁶ While such values derive from well considered calculations, it should be remembered that the variability in these estimates is trivial compared to the effects of a small variation in key parameters used to derive these estimates.

The observations that (i) Ca²⁺ puffs are rapidly evoked by photorelease of IP₃, with observed latencies [>250 ms⁴⁷], attributable to a local requirement for Ca²⁺ activation,⁴⁸ (ii) isolated Ca²⁺ blips are rare events,⁴¹ (iii) Ca²⁺ wavefronts are saltatory,^{47,49} reflecting progressive activation of proximal IP₃R clusters, and (iv) that the kinetic profiles Ca²⁺ puffs are similar in many diverse cell types expressing different IP₃R isoforms⁵⁰ support the concept that the basal architectural state for IP₃R organization comprises small clusters of IP₃Rs spaced throughout the ER. To the best of our knowledge, Ca²⁺ puff-like signals have not been resolved in invertebrate systems (but see Ref. 37), reflecting a general lack of insight into the molecular basis of IP₃R clustering behavior and the timing of its evolutionary emergence.

Therefore, Ca²⁺ puffs represent the basic building blocks from which whole cell Ca²⁺ signals are assembled. At higher levels of IP₃, Ca²⁺ puff frequency is increased and their

proximal activity coordinated by Ca²⁺ to repeatedly trigger Ca²⁺ waves that spread between neighboring Ca²⁺ release sites before inhibitory cues restrain Ca²⁺ release activity.^{48,51,52} Often, this behavior is entrained by the higher activity of unique Ca²⁺ puff sites that serve as the focus for Ca²⁺ wave initiation,^{52–54} demonstrating that IP₃R clusters in proximal ER regions can display different activities. The mechanistic basis for the higher IP₃R sensitivity at these sites remains unclear. Physiological cues control the positioning of the regenerative threshold relative to the size of the fundamental Ca²⁺ release event [Fig. 1(c)], thereby coordinating the predominance of local or global Ca²⁺ signaling domains with cellular physiology.^{1,55} In summary, this model derived from Ca²⁺ imaging data has had significant value in conceptualizing how Ca²⁺ waves trigger and propagate via summation of localized activity of clustered IP₃Rs.

A key caveat is that this interpretation entirely derives from functional measurements of open channels, reported via changes in the fluorescence of high affinity Ca²⁺ indicators with limited kinetic resolution. Therefore, while considerable effort has been spent, for example, (re)estimating the number of active channels during a Ca²⁺ puff, comparatively little is known about the underlying structural architecture of IP₃Rs beyond what has been inferred from divination of Ca²⁺ release profiles collected at high temporal resolution. Outstanding issues are insight into (i) the *total* number of IP₃Rs within a native cluster⁵⁶ (what is the open probability of an IP₃R during a Ca²⁺ puff?), (ii) cluster microarchitecture (how are IP₃R packed within a Ca²⁺ puff site?) and (iii) clustering mechanisms [what is the molecular basis of cluster (dis)assembly *in vivo*, the stoichiometry of accessory proteins if required, and by extension the mechanism that delimits native cluster size?]. These structural issues are interrelated, for example, observation of propagating “microwaves” within single Ca²⁺ puff sites^{49,57} and the observation of Ca²⁺ blip-like “triggers” preceding Ca²⁺ puffs⁴⁵ implies a small number of loosely corralled IP₃Rs [mean separation several fold greater than the lateral dimensions of a tetrameric IP₃R (Refs. 46, 58, and 59)] with relatively high open probability. Alternatively, clusters may consist of a higher number of more tightly packed channels (exemplified by the lateral arrays of IP₃Rs resolved in cerebellum⁶⁰) but with much lower likelihood of individual activation. IP₃R density also imposes spatial constraints on potential molecular mechanisms for cluster formation and functional recruitment, whether bridging via accessory proteins or direct interactions between adjacent IP₃R tetramers will suffice, and whether activation/inactivation is supported solely by Ca²⁺ feedback or by conformational spread between physically coupled Ca²⁺ adjacent channels.^{61–65} Finally, and possibly most importantly in terms of cellular physiology, there has been until recently⁶⁶ little experimental insight into the functional consequences of socialization into clusters on the unitary properties of the IP₃Rs (see Sec. IV and the review by Taylor’s group in this issue), let alone simultaneous structural/functional analyses of different IP₃R architectures within the same cell.

III. THE ORGANIZATION OF ER, AND IP₃RS WITHIN IT, IS MALLEABLE AND INDEPENDENT

Compartmentalization of Ca²⁺ signals results not only from static heterogeneities in the distribution of Ca²⁺-handling proteins but also from dynamic changes in the positioning of the ER, and via reorganization of IP₃Rs independently from changes in ER morphology. There is a growing awareness of the importance of these changes in patterning Ca²⁺ signals over physiologically relevant timescales, and consequently the need for realistic representation of this architecture in predictive models of Ca²⁺ dynamics.

ER dynamics. The ER is a highly dynamic organelle that adapts in organization both in response to cellular activity and preparatively for transitions in cellular physiology. Pathological cues also impact ER morphology, and consequently IP₃R localization.^{67,68} Morphological alterations of the ER can be dramatic (major repositioning,⁶⁹ reorganization,^{1,3,59,70,71} or more subtle alterations in the prevalence of subdomains that are constantly remodelling.^{72,73} Physiological⁷² (and protracted⁷⁴) changes in cytoplasmic Ca²⁺ concentration ([Ca²⁺]_{cyt}) regulate ER architecture by regulating a growing list of Ca²⁺-dependent effectors controlling ER structure,^{75,76} and reciprocally a changing ER architecture will impact cellular Ca²⁺ dynamics by redistributing ER-associated Ca²⁺ channels, pumps, and effectors throughout the cell.

IP₃R dynamics. While macroscopic reorganizations of the ER indirectly move IP₃Rs around the cell, changes in steady-state IP₃R localization^{14,31,77} and organization⁵² can occur independently of major alterations in ER structure, as also seen with other components of the cellular Ca²⁺ homeostatic machinery.^{78,79} Live cell imaging studies of fluorescent-protein tagged IP₃Rs have provided quantitative insight into the dynamics of IP₃Rs within the ER in live cells.^{80–82} Although it is appreciated that overexpressed IP₃Rs may not faithfully report native IP₃R behavior,^{80,83} it appears that the majority of cellular IP₃Rs are mobile and free to diffuse throughout the ER. Diffusibility (~100-fold range in estimates, Table I) is regulated by accessory proteins selective for specific IP₃R isoforms,⁸¹ and by interaction with other IP₃Rs.³³ Of particular relevance to the topic of this review is the observation originally made by Wilson *et al.*³² that endogenous IP₃Rs transiently aggregate during protracted stimulation into “larger IP₃R clusters” [average diameter, 0.35–2.3 μm (Ref. 84)] that then dissociate following agonist removal. Estimates of IP₃R diffusibility (Table I) are compatible with the observed density of IP₃R clusters: For example, in RBL-2H3 cells,³³ an IP₃R would diffuse through a surface area of ~30 μm² over a 10 min period, a territory likely containing several IP₃R aggregates (~1 per 10 μm²). This clustering behavior has been reported in several cell lines (Table II), and crucially is recapitulated when IP₃R constructs are overexpressed, thereby providing mechanistic insight into this phenomenon in live cells. Such studies^{33,84–86} have shown the following.

- (i) All IP₃R isoforms display activity-induced reorganization,⁸⁴ but cluster dimensions are shaped by

TABLE I. IP₃R mobility within the ER. Examples of FRAP-derived estimates for the diffusion coefficient and mobile fraction of fluorescent-protein tagged IP₃R_s in different cell types. Please refer to references for details of how these parameters were calculated in individual studies (n.r.=not reported).

Cell type	IP ₃ R isoform	Diffusion coefficient ($\mu\text{m}^2/\text{s}$)	Mobile fraction	Reference
MDCK (10B)	IP ₃ R1-GFP	0.004–0.01	100%, decreasing to 64% in bulk ER after polarization	82
CHO-K1	GFP-IP ₃ R3	0.031	67%	80
COS-7	GFP-IP ₃ R3	0.044	77%	80
RBL-2H3	YFP-IP ₃ R1	0.056	76%, decreasing to ~69% after IP ₃ R clustering	33
Hippocampal neurons	GFP-IP ₃ R1	0.26	n.r.	81
	GFP-IP ₃ R3	0.45		

the presence of specific IP₃R isoforms (but see Ref. 33).

- (ii) Cluster formation does not parallel major ER structural changes.^{33,85,86} Indeed, one report demonstrates IP₃R_s aggregate even within vesicularized ER (Ref. 85) (contrast with Ref. 86). Once formed, clusters are motile within contiguous ER and expand in size either via fusing with other clusters⁸⁶ or growing by further IP₃R entrapment.^{33,86}
- (iii) Protracted exposure to high concentrations of agonist, or IP₃,⁸⁵ is needed to induce IP₃R aggregation (Table II). Kinetically, this behavior occurs over a time frame of minutes, delayed [lags of ~60–90 s (Refs. 85 and 86)] to the initial agonist-evoked Ca²⁺ signal(s). Cluster disassembly occurs over a similar time frame, during⁸⁵ or following agonist removal,⁸⁶ likely by progressive attrition of clustered IP₃R. While this phenomenon has been extensively examined in two specific cell lines (Table II), it is less apparent in other cells without further manipulation of stored/extracellular Ca²⁺.⁸⁵ Given the intensity of cellular

stimulation required to trigger this behavior, the relationship of this mechanism to physiological “clustering” paradigms⁸⁷ and agonist-evoked Ca²⁺ spiking is unclear. For example, IP₃R1 and IP₃R3 are proposed to play opposing roles in facilitating and inhibiting Ca²⁺ oscillations,⁸⁸ but both form similar aggregates during stimulation.⁸⁴

- (iv) IP₃-evoked conformational change(s) is crucial in inducing higher-order IP₃R clustering. Binding-deficient mutants of individual IP₃R isoforms, as well as a natural binding-deficient IP₃R2 splice variant, fail to aggregate on cellular stimulation even as heteromultimers.^{84,86} More crucially, mutants competent at IP₃ binding but defective in the ensuing conformational movements that lead to channel activation fail to cluster. These mutational analyses represent a compelling argument that an IP₃-evoked conformational change—*independent of any Ca²⁺ flux through the channel*⁸⁶—controls IP₃R aggregation. Resolution of pre-existing clusters in unstimulated cells that endogenously^{32,33} or heterologously express IP₃R2

TABLE II. Activity-induced clustering of IP₃R_s in various cell lines. Summary of studies investigating the formation and properties of IP₃R clusters evoked by cellular stimulation. Readers are referred to indicated references for further experimental detail. Exogenous IP₃R constructs were expressed by transient transfection, unless indicated otherwise.

Cell type	IP ₃ R isoforms	Stimulus (cell surface)	Kinetic insight	Reference
RBL-2H3	Endogenous IP ₃ R2	Antigen, postpriming	Greater than three fold increase in number of IP ₃ R cluster within 10 min	32
RBL-2H3	Endogenous IP ₃ R2	Antigen	Clusters apparent after 30 min in periphery of permeabilized cells	108
RBL-2H3	Endogenous IP ₃ R2; Expressed YEP-IP ₃ R1	Antigen, postpriming	Increase in cluster size, not number (at least for endogenous IP ₃ R2) maximal by 10 min	33
COS-7	Expressed GFP-IP ₃ R1	ATP (10 μM)	Onset of ~1 min, progressive increase in number during agonist exposure, delayed relative to [Ca ²⁺] _{cyt} changes	86
COS-7	Expressed IP ₃ R1, IP ₃ R2 (SI _{m2+} , SI _{m2-}), IP ₃ R3	ATP (1 mM)	Analyzed after 20 min of ATP exposure. IP ₃ R2 (SI _{m2+}) clustered in unstimulated cells	84
COS-7	Expressed GFP-IP ₃ R3	ATP (100 μM)	Average onset of ~1 min, reversal within 20 min despite continued stimulation	85
HSY-EA1	Expressed GFP-IP ₃ R3 (stable cell line)	ATP (100 μM)	Clustering observed only in 10% of intact cells treated with ATP.	85
E36 ^{M3R}	Endogenous IP ₃ R2	Carbachol (1 mM)	Maximal after 30 min, reversed 30 min after agonist removal	
AR4-2J	Endogenous IP ₃ R2	CCK (0.5 μM)	Maximal by 30 min	32

(Ref. 84) has been suggested to relate to the higher IP₃ binding affinity of IP₃R2 relative to other IP₃R isoforms, such that ambient IP₃ levels are sufficient to adoption of a widespread, clustered architecture.⁸⁴

Understanding such a conformer-dependent reorganization necessitates definition of the relevant IP₃-induced conformational change within tetrameric IP₃R_s that promotes aggregation. Single particle analysis of IP₃R_s has revealed significant ligand-induced changes in IP₃R structure (albeit Ca²⁺-evoked changes⁸⁹) that would likely impact cluster microarchitecture depending on the state of channel activation. For example, the transition between “compact” and “windmill” IP₃R structures⁸⁹ is striking (~1.5-fold change in diameter/cross sectional surface area), and may be of sufficient magnitude to impose conformational restrictions on activation, if packing density is too high, or inactivation and resensitization if active conformers sterically trap neighbors in conformations (“conformational locking”) that persist beyond ligand dissociation from individual channels. Consequently, the interrelationship between agonist occupancy, efficacy at promoting conformational changes and the ensuing initiation and reversal of clustering requires further experimental and modeling insight.

IV. IP₃RS ARE GREGARIOUS, BUT THE FUNCTIONAL RAMIFICATIONS OF SOCIALIZATION APPEAR BIPHASIC

From the preceding discussion, it is clear that cellular IP₃R_s are gregarious and dynamically reorganize between heterogeneous populations of “clustered” architectures. Is IP₃R functionality regulated by this reorganization? Experimental studies with other membrane proteins have demonstrated that clustering regulates signaling activity, and reciprocally, signaling activity impacts clustering.^{90–96} This reciprocity ensures diversity in cellular receptor architectures such that—as with IP₃R_s—native clusters of receptors, often too small to be resolved by confocal microscopy, are distinct from activity-induced aggregates. Functionally, the effects of receptor clustering can be manifest as either social excitability or inhibition, achieved through modulation of ligand sensitivity, channel transition rates, dynamic range, and the spatial spread of information at a cellular level.^{90,97–100} Proposed mechanisms underpinning differential receptor behavior in clusters expand beyond effects of scaling in terms of increased receptor number and encompass the potential for amplification via activity-spread throughout the cluster, changes in the probability or kinetics of ligand (re)binding/dissociation consequent to clustering, or altered ligand avidity owing to the adoption of a distinct receptor conformation or microarchitecture as a prerequisite for cluster formation. For IP₃R_s, feedback regulation by Ca²⁺ provides an additional level of interchannel regulation that, on the basis of quantitative modeling studies, has been shown to vary for different cluster microarchitectures,^{64,65,101} and more generally as a consequence of clustering itself⁶⁶ (see also review by Taylor’s group, this issue).

Therefore, in terms of terminology, it is clear that cluster has been used as a qualitative catch-all in the IP₃R literature

to describe a broad scale of native and activity-induced IP₃R architectures. Although this descriptive sleight covers our lack of knowledge of microscopic IP₃R organization, it is problematic as it likely collates distinct basal and adaptive IP₃R architectures with divergent functional properties. Although this issue has not been directly addressed experimentally (e.g., by simultaneously imaging the structure and functionality of tagged IP₃R_s), several pieces of data suggest that large scale aggregation attenuates IP₃R activity. First, the stimulation paradigms that induce IP₃R aggregation require protracted exposure to high agonist concentrations (Table II) and consequently aggregation lags initial agonist-evoked Ca²⁺ signals.^{33,85,86} This likely correlates with depleted ER Ca²⁺ stores (addition of Ca²⁺ to permeabilized cells prevents IP₃R aggregation⁸⁵), a condition known to decrease IP₃R sensitivity.¹⁰² Notably, the duration of photolysis-evoked Ca²⁺ transients is reduced following formation of IP₃R aggregates in RBL cells.¹⁰¹ Second, ERp44 targets to IP₃R1 aggregates.⁸⁶ This ER luminal protein inhibits the activity of the ubiquitously expressed IP₃R1.^{103,104} Third, most generally, other scenarios of increased IP₃R density are associated with decreased IP₃R sensitivity. Specialized regions of the ER in oocytes with high IP₃R density^{1,59} show attenuated local Ca²⁺ release activity until these domains are physiologically remodeled during oocyte maturation. In cerebellar Purkinje cells, electron microscopy-level resolution of ER regions with high IP₃R density^{9,60,105} (approximately ten times density of adjacent ER,⁹ or as physically coupled IP₃R arrays⁶⁰) that increase during hypoxia is speculated to be an adaptation to decrease ER Ca²⁺ efflux. Collectively, such data suggest high density packing of IP₃R_s or activity-induced aggregation suppresses IP₃R activity.^{1,85,101} Stochastic and deterministic modeling analyses also support this contention, justifying a conceptual separation of native, IP₃R clusters, optimized for generating the hierarchical diversity of Ca²⁺ signals,^{106,107} from higher order IP₃R “aggregates” that attenuate Ca²⁺ release from the ER.¹⁰¹ Although the functionality at the extremes (clusters versus aggregates) is different, the formation of these structures may nevertheless be underpinned by a conserved mechanism (IP₃-evoked IP₃R clustering) that spans the architectural continuum.

Therefore, the Ca²⁺ releasing ability of clusters may not equate with a proportional scaling of single channel activity (as assumed in many modeling analyses), rather IP₃R socialization may lead first to a disproportional facilitation of Ca²⁺ release activity in small IP₃R clusters that generate Ca²⁺ puffs, and then repression of IP₃R responsiveness in larger, activity-induced IP₃R aggregates (Fig. 2). Little is known quantitatively about transitions between these states and clearly further experimental and modeling insight is needed. At odds with this model is that suggestion that IP₃R2(SI_{m2+}) clusters, observed in resting COS-7 cells, represent native Ca²⁺ puff sites. Although their dimensions are smaller (greater than fourfold) than clusters formed by IP₃R1 or IP₃R3 in stimulated cells, this proposal appears premature in the absence of simultaneous structural/functional measurement. IP₃R1 and IP₃R3 do not form similar clusters in resting cells,⁸⁴ but Ca²⁺ puff kinetics are similar in cells enriched in these different IP₃R isoforms.⁵⁰

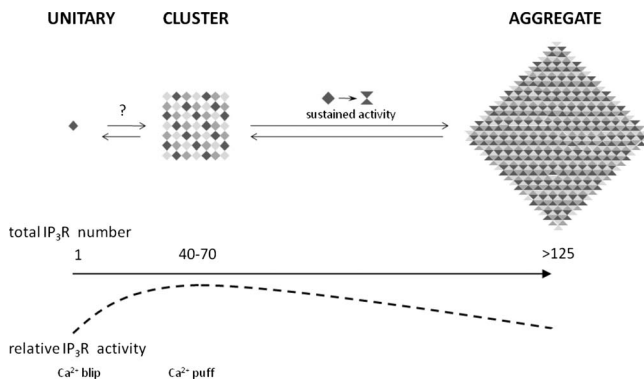


FIG. 2. Functional architecture of IP₃R—structural and functional distinction of native clusters and activity-induced aggregates. IP₃R socialization from (i) “unitary” channels to (ii) native clusters that support Ca²⁺ puffs to (iii) larger IP₃R aggregates observed following sustained stimulation. While little is known about mechanisms regulating cluster assembly, a change in IP₃R conformation (e.g., diamond to hourglass) is a prerequisite for aggregate formation. The functional effects of IP₃R socialization may not scale linearly with channel number, rather different architectures reflect distinct states of IP₃R activity: native “loose” clustering promotes IP₃R activity, whereas high density packing or activity-induced aggregation attenuates IP₃R activity. Numerical estimates are from Refs. 45, 56, and 101. For clarity, individual tetramers are represented in different shades of gray.

V. DYNAMIC IP₃R ARCHITECTURES, REALISTIC CELLULAR GEOMETRIES: RAMIFICATIONS FOR MODELLING

Experimental data have resolved a broad scope of cellular IP₃R architectures (from isolated channels to a heterogeneous population of higher-order structures exhibiting different organizations and functionalities). Appreciation of the diversity and dynamic malleability of IP₃R organization, and of the ER itself, poses a considerable challenge for *in silico* modelling. For reasons of obvious simplification, studies are frequently based on dimensionless representations of monotypic IP₃R clusters within gridded, static, planar arrays. Clearly, this neither represents *in vivo* IP₃R dynamics nor implicitly considers the effect of IP₃R organization on IP₃R behavior. Modelling an IP₃R cluster either as a collective of functionally independently channels or as an extrapolated multiple of the properties of an isolated IP₃R, while iterative, has been proven precariously assumptive especially in the absence of experimental insight into the ultrastructural basis of local Ca²⁺ signals. Although this broad architecture theme is not new (judiciously addressed over a decade ago in terms of ultrastructural limitations for channel synchronization by Ca²⁺-induced Ca²⁺ release rather than conformational coupling⁶⁴), its importance is being resuscitated by recent computational analyses.^{35,46,65,101} Especially significant is the transition toward modelling Ca²⁺ channel architectures within realistic cellular geometries.^{35,101} Notable, in the context of this review, are results such as those reported by Means *et al.*¹⁰¹ that address the functional effects of transitions between different IP₃R architectures, including analyses within a realistic rendering of ER morphology. Stochastic and deterministic simulations comparing two states—diffuse versus aggregates observed on protracted stimulation (predicted to comprise of ~125 IP₃R2 channels)—suggest that aggregation reduces IP₃R open probability and the rate of

Ca²⁺ release from the ER as a result of differential local Ca²⁺ feedback regulation. In this study, social inhibition of proximal IP₃R was predicted even in loosely coupled aggregates (more analogous to Ca²⁺ puff architecture) albeit only from deterministic openings of a small proportion (<3%) of colocalized IP₃R. Clearly, more extensive simulations are required, but incorporation of biologically relevant architectures^{35,101} is a major step forward toward predictive models of macroscopic Ca²⁺ dynamics. Future studies will likely incorporate IP₃R dynamics and the real-time (dis)assembly of different IP₃R clustered architectures, as well as physiological relevant changes in ER organization and Ca²⁺ homeostasis.

In summary, it is clear that cellular IP₃R architectures are dynamic and diverse. Comparatively little quantitative insight is available regarding the endogenous ultrastructural organization of IP₃R or the functional impact of these different IP₃R architectures in gearing IP₃R responsiveness. However, the ability of cells to remodel IP₃R into architectures with different functionalities likely provides flexibility in adapting to physiological cues and withstanding pathological insults. Appreciation of this functional architecture of IP₃R is therefore essential in developing models to faithfully capture the full range of ER Ca²⁺ release profiles in any given cell type.

ACKNOWLEDGMENTS

Work is supported by NIH (Contract No. GM088790) and NSF (J.S.M.) and CONICET-Argentina (L.D.).

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