Localization and socialization: Experimental insights into the functional architecture of IP$_3$ receptors

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Inositol 1,4,5-trisphosphate (IP$_3$)-evoked Ca$^{2+}$ signals display great spatiotemporal malleability. This malleability depends on diversity in both the cellular organization and in situ functionality of IP$_3$ receptors (IP$_3$Rs) that regulate Ca$^{2+}$ 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 IP$_3$Rs impacts their functionality, and reciprocally IP$_3$R activity impacts their organization within native ER membranes. Here, we (i) review experimental data that lead to our understanding of the “functional architecture” of IP$_3$Rs within the ER, (ii) propose an updated terminology to span the organizational hierarchy of IP$_3$Rs observed in intact cells, and (iii) speculate on the physiological significance of IP$_3$R socialization in Ca$^{2+}$ dynamics, and consequently the emerging need for modeling studies to move beyond gridded, planar, and static simulations of IP$_3$R clustering even over short experimental timescales. © 2009 American Institute of Physics. [DOI: 10.1063/1.3147425]

Changes in cytoplasmic Ca$^{2+}$ concentration control the activity of numerous cellular proteins. Inositol 1,4,5-trisphosphate receptors (IP$_3$Rs) localized on the endoplasmic reticulum play a key role in regulating cellular Ca$^{2+}$ homeostasis by controlling Ca$^{2+}$ flux into the cytoplasm from this intracellular Ca$^{2+}$ store. IP$_3$R stimulation results in complex spatial and temporal patterns of intracellular Ca$^{2+}$ release activity. Consequently, the utility of computational approaches in recapitulating and predicting Ca$^{2+}$ release dynamics requires accurate definition of the subcellular dynamics and in situ functionality of cellular IP$_3$Rs. This review summarizes experimental data underpinning our current understanding of the functional architecture of IP$_3$Rs in the endoplasmic reticulum, to highlight the need for the next generation of modeling studies to consider the functional ramifications of IP$_3$R socialization within diverse and dynamic architectures in live cells.

I. INTRODUCTION

The cellular architecture of IP$_3$Rs is diverse: Their global distribution is not uniform, and their localized organization is not stereotypic. This organizational diversity is significant for patterning Ca$^{2+}$ transients, crudely by dictating the targeting and propagation of Ca$^{2+}$ signals (“localization”), but likely also by impacting the functional properties of IP$_3$Rs within the native membranes where they reside (“socialization”). Therefore, from an experimentalist’s perspective, predictive modelling of cellular Ca$^{2+}$ signals necessitates dissecting the “functional architecture” of IP$_3$Rs, 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 IP$_3$Rs from the macroscopic (cellular, “global”) down to the microscopic (subcellular, “local”) scale, ensuing ramifications for IP$_3$R function, and emerging modeling attempts to predict the dynamics and properties of IP$_3$Rs in different states of organization within realistic cellular geometries.

II. THE GLOBAL DISTRIBUTION AND LOCAL ORGANIZATION OF IP$_3$Rs IS INHOMOGENOUS

There can be considerable macroscopic heterogeneity in IP$_3$R 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 IP$_3$R localization in polarized cells demonstrated by antibody/fluorescent ligand labeling, fractionation, and functional Ca$^{2+}$ imaging approaches, as well by resolution in electron micrographs of variable IP$_3$R densities between different subcellular regions of ER. Similarly, targeting of IP$_3$R outside the “bulk” endoplasmic reticulum—at the cell surface, in other organelles, or within deep nuclear projections—may further bias IP$_3$R distribution across the cell. Such gradients in endogenous IP$_3$R localization are crucial for cellular physiology by impacting resting Ca$^{2+}$ gradients, as well as the initiation and the spatiotemporal dynamics of agonist-evoked Ca$^{2+}$ signals. Often these differential distributions derive from differential targeting of IP$_3$R isoforms, but regionalization also occurs in cells where a single IP$_3$R iso-
form predominates (for example, Refs. 1, 9, and 16–18). Indeed, there is growing molecular insight into mechanisms dictating IP$_3$R localization in diverse cell types.$^{2,19,20}$ While, obviously, graded spatial expression is but one way to variegate intracellular Ca$^{2+}$ release activity, it provides a simple route to customize cellular Ca$^{2+}$ signals.

Similarly, at the microscopic level, the local organization of IP$_3$Rs within the ER is not homogenous. First, in peripheral ER closely juxtaposed to the cell surface, IP$_3$Rs frequently show punctate distributions within signaling complexes, localized by molecular interactions unique at this interface. The existence of such IP$_3$R-enriched “micro-domains” identified in diverse cell types, serve to define agonist specificity and/or initiation sites of Ca$^{2+}$ signals.$^{27-28}$

Within nonperipheral ER, both structural and functional data suggest that IP$_3$Rs are gregarious, organizing into discrete “clusters” in “unstimulated” cells. For example, antibody staining reveals punctate IP$_3$R immunoreactivity throughout the ER in primary [Fig. 1(a)] (Refs. 16 and 29–31) and immortaliized 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 Ca$^{2+}$ channel clusters,$^{34,35}$ such methods have not yet been applied to IP$_3$Rs. Consequently, the majority of evidence supporting a native, clustered organization of IP$_3$Rs derives from functional rather than structural measurements, specifically electrophysiological analyses of endogenous IP$_3$R distributions in excised nuclei$^{36,37}$ and predominantly from confocal Ca$^{2+}$ imaging in intact cells.$^{38,39}$

Rapid, confocal Ca$^{2+}$ imaging methods revealed that IP$_3$-evoked Ca$^{2+}$ release from the ER was a discontinuous process, by demonstrating discrete and recurrent sites of Ca$^{2+}$ release spaced throughout the ER. At low levels of stimulation, localized Ca$^{2+}$ 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 Ca$^{2+}$ release events were christened Ca$^{2+}$ “puffs”$^{40}$ and combined experimental and modeling analyses of Ca$^{2+}$ puff properties have established that they result from the coordinated opening of a small number of Ca$^{2+}$ channels.$^{41,42}$ Similar “elementary” responses mediated by clusters of ryanodine receptors, the other major class of intracellular Ca$^{2+}$ channels are discussed elsewhere.$^{43,44}$ The variabilities in the amplitude of successive Ca$^{2+}$ puff events and peak amplitude of Ca$^{2+}$ puffs resolved at the same site suggest that not all IP$_3$Rs open during a Ca$^{2+}$ puff and that the underlying number of clustered receptors varies between Ca$^{2+}$ release sites.$^{41,42}$ A recent experimental estimate, calibrating probable single channel responses (“Ca$^{2+}$ blips”) to subsequent Ca$^{2+}$ puffs that they rapidly trigger suggest between ~6 and 60 IP$_3$Rs open during a Ca$^{2+}$ puff.$^{45}$ Deterministic simulations of the fluorescence profiles of these coupled events further refine these experimental estimates to suggest that Ca$^{2+}$ puffs could result from the activity of ~25–35 IP$_3$Rs 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) Ca$^{2+}$ puffs are rapidly evoked by photorelease of IP$_3$, with observed latencies [>250 ms$^{47}$], attributable to a local requirement for Ca$^{2+}$ activation,$^{48}$ (ii) isolated Ca$^{2+}$ blips are rare events,$^{41}$ (iii) Ca$^{2+}$ wavefronts are salutary,$^{47,49}$ reflecting progressive activation of proximal IP$_3$R clusters, and (iv) that the kinetic profiles Ca$^{2+}$ puffs are similar in many diverse cell types expressing different IP$_3$R isoforms$^{50}$ support the concept that the basal architectural state for IP$_3$R organization comprises small clusters of IP$_3$Rs spaced throughout the ER. To the best of our knowledge, Ca$^{2+}$ 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 IP$_3$R clustering behavior and the timing of its evolutionary emergence.

Therefore, Ca$^{2+}$ puffs represent the basic building blocks from which whole cell Ca$^{2+}$ signals are assembled. At higher levels of IP$_3$, Ca$^{2+}$ puff frequency is increased and their
proximal activity coordinated by Ca\textsuperscript{2+} to repeatedly trigger Ca\textsuperscript{2+} waves that spread between neighboring Ca\textsuperscript{2+} release sites before inhibitory cues restrain Ca\textsuperscript{2+} release activity.\textsuperscript{48,51,52} Often, this behavior is entrained by the higher activity of unique Ca\textsuperscript{2+} puff sites that serve as the focus for Ca\textsuperscript{2+} wave initiation,\textsuperscript{52–54} demonstrating that IP\textsubscript{3}Rs clusters in proximal ER regions can display different activities. The mechanistic basis for the higher IP\textsubscript{3}R sensitivity at these sites remains unclear. Physiological cues control the positioning of the regenerative threshold relative to the size of the fundamental Ca\textsuperscript{2+} release event [Fig. 1(c)], thereby coordinating the predominance of local or global Ca\textsuperscript{2+} signaling domains with cellular physiology.\textsuperscript{1,55} In summary, this model derived from Ca\textsuperscript{2+} imaging data has had significant value in conceptualizing how Ca\textsuperscript{2+} waves trigger and propagate via summation of localized activity of clustered IP\textsubscript{3}Rs.

A key caveat is that this interpretation entirely derives from functional measurements of open channels, reported via changes in the fluorescence of high affinity Ca\textsuperscript{2+} indicators with limited kinetic resolution. Therefore, while considerable effort has been spent, for example, (re)estimating the number of active channels during a Ca\textsuperscript{2+} puff, comparatively little is known about the underlying structural architecture of IP\textsubscript{3}R\textsubscript{s} beyond what has been inferred from divination of Ca\textsuperscript{2+} release profiles collected at high temporal resolution. Outstanding issues are insight into (i) the total number of IP\textsubscript{3}R\textsubscript{s} within a native cluster\textsuperscript{56} (what is the open probability of an IP\textsubscript{3}R during a Ca\textsuperscript{2+} puff?), (ii) cluster microarchitecture (how are IP\textsubscript{3}Rs packed within a Ca\textsuperscript{2+} puff site?) and (iii) clustering mechanisms (what is the molecular basis of cluster (dis)assembly \textit{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 Ca\textsuperscript{2+} puff sites\textsuperscript{49,57} and the observation of Ca\textsuperscript{2+} blip-like “triggers” preceding Ca\textsuperscript{2+} puffs\textsuperscript{45} implies a small number of loosely corralled IP\textsubscript{3}Rs [mean separation several fold greater than the lateral dimensions of a tetrameric IP\textsubscript{3}R (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 IP\textsubscript{3}R\textsubscript{s} resolved in cerebellum\textsuperscript{60}) but with much lower likelihood of individual activation. IP\textsubscript{3}R 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 IP\textsubscript{3}R tetramers will suffice, and whether activation/inactivation is supported solely by Ca\textsuperscript{2+} feedback or by conformational spread between physically coupled Ca\textsuperscript{2+} adjacent channels\textsuperscript{61–65} Finally, and possibly most importantly in terms of cellular physiology, there has been until recently\textsuperscript{66} little experimental insight into the functional consequences of socialization into clusters on the unitary properties of the IP\textsubscript{3}Rs (see Sec. IV and the review by Taylor’s group in this issue), let alone simultaneous structural-functional analyses of different IP\textsubscript{3}R architectures within the same cell.

III. THE ORGANIZATION OF ER, AND IP\textsubscript{3}RS WITHIN IT, IS MALLEABLE AND INDEPENDENT

Compartmentalization of Ca\textsuperscript{2+} signals results not only from static heterogeneities in the distribution of Ca\textsuperscript{2+}-handling proteins but also from dynamic changes in the positioning of the ER, and via reorganization of IP\textsubscript{3}R\textsubscript{s} independently from changes in ER morphology. There is a growing awareness of the importance of these changes in patterning Ca\textsuperscript{2+} signals over physiologically relevant timescales, and consequently the need for realistic representation of this architecture in predictive models of Ca\textsuperscript{2+} dynamics.

\textit{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 IP\textsubscript{3}R localization.\textsuperscript{67,68} Morphological alterations of the ER can be dramatic (major repositioning,\textsuperscript{69} reorganization,\textsuperscript{1,3,59,70,71} or more subtle alterations in the prevalence of subdomains that are constantly remodelling,\textsuperscript{72,73} Physiological (and protracted\textsuperscript{74} changes in cytoplasmic Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{cyt}) regulate ER architecture by regulating a growing list of Ca\textsuperscript{2+}-dependent effectors controlling ER structure,\textsuperscript{75,76} and reciprocally a changing ER architecture will impact cellular Ca\textsuperscript{2+} dynamics by redistributing ER-associated Ca\textsuperscript{2+} channels, pumps, and effectors throughout the cell.

\textit{IP\textsubscript{3}R dynamics.} While macroscopic reorganizations of the ER indirectly move IP\textsubscript{3}Rs around the cell, changes in steady-state IP\textsubscript{3}R localization\textsuperscript{14,31,77} and organization\textsuperscript{52} can occur independently of major alterations in ER structure, as also seen with other components of the cellular Ca\textsuperscript{2+} homeostatic machinery.\textsuperscript{78,79} Live cell imaging studies of fluorescent-protein tagged IP\textsubscript{3}R\textsubscript{s} have provided quantitative insight into the dynamics of IP\textsubscript{3}R\textsubscript{s} within the ER in live cells.\textsuperscript{80–82} Although it is appreciated that overexpressed IP\textsubscript{3}R\textsubscript{s} may not faithfully report native IP\textsubscript{3}R behavior,\textsuperscript{80,83} it appears that the majority of cellular IP\textsubscript{3}R\textsubscript{s} 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 IP\textsubscript{3}R isoforms,\textsuperscript{81} and by interaction with other IP\textsubscript{3}R\textsubscript{s}.\textsuperscript{83} Of particular relevance to the topic of this review is the observation originally made by Wilson \textit{et al.}\textsuperscript{32} that endogenous IP\textsubscript{3}R\textsubscript{s} transiently aggregate during protracted stimulation into “larger IP\textsubscript{3}R clusters” [average diameter, 0.35–2.3 μm (Ref. 84)] that then dissociate following agonist removal. Estimates of IP\textsubscript{3}R diffusibility (Table I) are compatible with the observed density of IP\textsubscript{3}R clusters: For example, in RBL-2H3 cells,\textsuperscript{33} an IP\textsubscript{3}R would diffuse through a surface area of ∼30 μm\textsuperscript{2} over a 10 min period, a territory likely containing several IP\textsubscript{3}R aggregates (∼1 per 10 μm\textsuperscript{2}). This clustering behavior has been reported in several cell lines (Table II), and crucially is recapitulated when IP\textsubscript{3}R constructs are overexpressed, thereby providing mechanistic insight into this phenomenon in live cells. Such studies\textsuperscript{33,84–86} have shown the following.

(i) All IP\textsubscript{3}R isoforms display activity-induced reorganization,\textsuperscript{84} but cluster dimensions are shaped by
the presence of specific IP$_3$R isoforms (but see Ref. 33).

(ii) Cluster formation does not parallel major ER structural changes. Indeed, one report demonstrates IP$_3$Rs aggregate even within vesicularized ER (Ref. 85) (contrast with Ref. 86). Once formed, clusters are mobile within contiguous ER and expand in size either via fusing with other clusters or growing by further IP$_3$R entrapment.

(iii) Protracted exposure to high concentrations of agonist, or IP$_3$, is needed to induce IP$_3$R 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 Ca$^{2+}$ signal(s). Cluster disassembly occurs over a similar time frame, during or following agonist removal, likely by progressive attrition of clustered IP$_3$R. 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 Ca$^{2+}$. Given the intensity of cellular stimulation required to trigger this behavior, the relationship of this mechanism to physiological “clustering” paradigms and agonist-evoked Ca$^{2+}$ spiking is unclear. For example, IP$_3$R1 and IP$_3$R3 are proposed to play opposing roles in facilitating and inhibiting Ca$^{2+}$ oscillations, but both form similar aggregates during stimulation.

(iv) IP$_3$-evoked conformational change(s) is crucial in inducing higher-order IP$_3$R clustering. Binding-deficient mutants of individual IP$_3$R isoforms, as well as a natural binding-deficient IP$_3$R2 splice variant, fail to aggregate on cellular stimulation even as heteromultimers. More crucially, mutants competent at IP$_3$ 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 IP$_3$-evoked conformational change—indeed of any Ca$^{2+}$ flux through the channel—controls IP$_3$R aggregation. Resolution of pre-existing clusters in unstimulated cells that endogenously or heterologously express IP$_3$R2

<table>
<thead>
<tr>
<th>Cell type</th>
<th>IP$_3$R isoforms</th>
<th>Stimulus (cell surface)</th>
<th>Kinetic insight</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBL-2H3</td>
<td>Endogenous IP$_3$R2</td>
<td>Antigen, postpriming</td>
<td>Greater than three fold increase in number of IP$_3$R clusters, reversal within 10 min</td>
<td>32</td>
</tr>
<tr>
<td>RBL-2H3</td>
<td>Endogenous IP$_3$R2</td>
<td>Antigen</td>
<td>Clusters apparent after 30 min in periphery of permeabilized cells</td>
<td>108</td>
</tr>
<tr>
<td>RBL-2H3</td>
<td>Endogenous IP$_3$R2, Expressed YEP-IP$_3$R1</td>
<td>Antigen, postpriming</td>
<td>Increase in cluster size, not number (at least for endogenous IP$_3$R2) maximal by 10 min</td>
<td>33</td>
</tr>
<tr>
<td>COS-7</td>
<td>Expressed GFP-IP$_3$R1</td>
<td>ATP (10 μM)</td>
<td>Onset of ~1 min, progressive increase in number during agonist exposure, delayed relative to [Ca$^{2+}]_{cyt}$ changes</td>
<td>86</td>
</tr>
<tr>
<td>COS-7</td>
<td>Expressed IP$<em>3$R1, IP$<em>3$R2 (SL$2</em>{17}$, SL$2</em>{18}$), IP$_3$R3</td>
<td>ATP (1 mM)</td>
<td>Analyzed after 20 min of ATP exposure. IP$<em>3$R2 (SL$2</em>{17}$) clustered in unstimulated cells</td>
<td>84</td>
</tr>
<tr>
<td>COS-7</td>
<td>Expressed GFP-IP$_3$R3</td>
<td>ATP (100 μM)</td>
<td>Average onset of ~1 min, reversal within 20 min despite continued stimulation</td>
<td>85</td>
</tr>
<tr>
<td>HSY-EA1</td>
<td>Expressed GFP-IP$_3$R3 (stable cell line)</td>
<td>ATP (100 μM)</td>
<td>Clustering observed only in 10% of intact cells treated with ATP.</td>
<td>85</td>
</tr>
<tr>
<td>E36$^{MR}$</td>
<td>Endogenous IP$_3$R2</td>
<td>Carbachol (1 mM)</td>
<td>Maximal after 30 min, reversed 30 min after agonist removal</td>
<td>32</td>
</tr>
<tr>
<td>AR4-2J</td>
<td>Endogenous IP$_3$R2</td>
<td>CCK (0.5 μM)</td>
<td>Maximal by 30 min</td>
<td>32</td>
</tr>
</tbody>
</table>

TABLE II. Activity-induced clustering of IP$_3$Rs in various cell lines. Summary of studies investigating the formation and properties of IP$_3$R clusters evoked by cellular stimulation. Readers are referred to indicated references for further experimental detail. Exogenous IP$_3$R constructs were expressed by transient transfection, unless indicated otherwise.
(Ref. 84) has been suggested to relate to the higher IP$_3$ binding affinity of IP$_3$R2 relative to other IP$_3$R isoforms, such that ambient IP$_3$ levels are sufficient to adoption of a widespread, clustered architecture.84

Understanding such a conformer-dependent reorganization necessitates definition of the relevant IP$_3$-induced conformational change within tetrameric IP$_3$Rs that promotes aggregation. Single particle analysis of IP$_3$Rs has revealed significant ligand-induced changes in IP$_3$R structure (albeit Ca$^{2+}$-evoked changes 89) that would likely impact cluster microarchitecture depending on the state of channel activation. For example, the transition between “compact” and “windmill” IP$_3$R structures 89 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 desensitization 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. IP$_3$Rs ARE GREGARIOUS, BUT THE FUNCTIONAL RAMIFICATIONS OF SOCIALIZATION APPEAR BIPHASIC

From the preceding discussion, it is clear that cellular IP$_3$Rs are gregarious and dynamically reorganize between heterogeneous populations of “clustered” architectures. Is IP$_3$Rs 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 IP$_3$Rs—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.90–96 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 alerted ligand avidity owing to the adoption of a distinct receptor conformation or microarchitecture as a prerequisite for cluster formation. For IP$_3$Rs, feedback regulation by Ca$^{2+}$ 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 itself 66 (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 IP$_3$R literature to describe a broad scale of native and activity-induced IP$_3$R architectures. Although this descriptive sleight covers our lack of knowledge of microscopic IP$_3$R organization, it is problematic as it likely collates distinct basal and adaptive IP$_3$R 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 IP$_3$Rs), several pieces of data suggest that large scale aggregation attenuates IP$_3$R activity. First, the stimulation paradigms that induce IP$_3$R aggregation require protracted exposure to high agonist concentrations (Table II) and consequently aggregation lags initial agonist-evoked Ca$^{2+}$ signals.53,85,86 This likely correlates with depleted ER Ca$^{2+}$ stores (addition of Ca$^{2+}$ to permeabilized cells prevents IP$_3$R aggregation 85), a condition known to decrease IP$_3$R sensitivity.102 Notably, the duration of photolysis-evoked Ca$^{2+}$ transients is reduced following formation of IP$_3$R aggregates in RBL cells.101 Second, ERP44 targets to IP$_3$R aggregates.85 This ER lumenal protein inhibits the activity of the ubiquitously expressed IP$_3$R1103,104 Third, most generally, other scenarios of increased IP$_3$R density are associated with decreased IP$_3$R sensitivity. Specialized regions of the ER in oocytes with high IP$_3$R density 159 show attenuated local Ca$^{2+}$ release activity until these domains are physiologically remodeled during oocyte maturation. In cerebellar Purkinje cells, electron microscopy-level resolution of ER regions with high IP$_3$R density 5,60,105 (approximately ten times density of adjacent ER,1 or as physically coupled IP$_3$R arrays60), that increase during hypoxia is speculated to be an adaptation to decrease ER Ca$^{2+}$ efflux. Collectively, such data suggest high density packing of IP$_3$Rs or activity-induced aggregation suppresses IP$_3$R activity.1,85,101 Stochastic and deterministic modeling analyses also support this contention, justifying a conceptual separation of native, IP$_3$R clusters, optimized for generating the hierarchical diversity of Ca$^{2+}$ signals, from higher order IP$_3$R “aggregates” that attenuate Ca$^{2+}$ 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 (IP$_3$-evoked IP$_3$R clustering) that spans the architectural continuum.

Therefore, the Ca$^{2+}$ releasing ability of clusters may not equate with a proportional scaling of single channel activity (as assumed in many modeling analyses), rather IP$_3$R socialization may lead first to a disproportional facilitation of Ca$^{2+}$ release activity in small IP$_3$R clusters that generate Ca$^{2+}$ puffs, and then repression of IP$_3$R responsiveness in larger, activity-induced IP$_3$R 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 IP$_3$R2(SL$_{IP3}$R2) clusters, observed in resting COS-7 cells, represent native Ca$^{2+}$ puff sites. Although their dimensions are smaller (greater than fourfold) than clusters formed by IP$_3$R1 or IP$_3$R3 in stimulated cells, this proposal appears premature in the absence of simultaneous structural/functional measurement. IP$_3$R1 and IP$_3$R3 do not form similar clusters in resting cells,84 but Ca$^{2+}$ puff kinetics are similar in cells enriched in these different IP$_3$R isoforms.60
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 coupling[64]), its importance is being resuscitated by recent computational analyses.[3,5,6,8,10,11] Especially significant is the transition toward modelling Ca²⁺ channel architectures within realistic cellular geometries.[15,101] Notable, in the context of this review, are results such as those reported by Means et al.[10] 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 IP₃Rs 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 IP₃Rs. 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 IP₃Rs or the functional impact of these different IP₃R architectures in gearing IP₃R responsiveness. However, the ability of cells to remodel IP₃Rs into architectures with different functionalties likely provides flexibility in adapting to physiological cues and withstand pathological insults. Appreciation of this functional architecture of IP₃Rs is therefore essential in developing models to faithfully capture the full range of ER Ca²⁺ release profiles in any given cell type.

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