Themes and Variations in ER/SR Calcium Release Channels: Structure and Function

Calcium (Ca$^{2+}$) release from reticular stores is a vital regulatory signal in eukaryotes. Recent structural data on large NH$_2$-terminal regions of IP$_3$Rs and RyRs and their tetrameric arrangement in the full-length context reveal striking mechanistic similarities in Ca$^{2+}$ release channel function. A common ancestor found in unicellular genomes underscores the fundamentality of these elements to Ca$^{2+}$ release channels.

The calcium (Ca$^{2+}$) ion is a universal messenger that controls a vast number of cellular processes such as the prolonged regulation of transcription, cell division, and apoptosis, as well as the more short-lived secretion and contraction (5, 6). The Ca$^{2+}$ ion plays an indispensible role in contractile, neuronal as well as nonexcitable cells (4–6, 133, 136, 137). At rest, cells compartmentalize most intracellular Ca$^{2+}$ ([Ca$^{2+}$]$\_i$) in the sarco-/endoplasmic reticulum (SR/ER) stores. Upon stimulation, Ca$^{2+}$ is released from the SR/ER into the intracellular space (i.e., cytosol); the spatial and temporal release and uptake of [Ca$^{2+}$]$\_i$ is the driving force behind these myriad cellular processes in health and disease.

There are two types of Ca$^{2+}$ handling macromolecules on the SR/ER membrane: first, Ca$^{2+}$ release receptor-operated channels (ROCs) liberate internally stored Ca$^{2+}$ into the cytoplasm; second, Ca$^{2+}$ pumps replete the internal store of Ca$^{2+}$. The SR/ER Ca$^{2+}$ ATPase (SERCA) pumps are a major family of SR/ER-residing Ca$^{2+}$ pumps that have been the topic of review in the past (82, 131). The two major groups of Ca$^{2+}$ release channels include the ubiquitously expressed inositol 1,4,5-trisphosphate receptors (IP$_3$Rs) and the ryanodine receptors (RyRs) that find more exclusively in excitable cells. These ROCs belong to a voltage-independent class of Ca$^{2+}$ channels; nonetheless, RyR function is intimately tied to voltage-operated channels (VOCs) in various cell types through excitation-contraction coupling and Ca$^{2+}$-induced Ca$^{2+}$ release (CICR). Although RyR channels can be regulated by ryanodine binding (i.e., ~nM concentrations open the channel, whereas sub-nM concentrations inhibit the channel) (80), Ca$^{2+}$ binding itself triggers RyR channel opening, and RyRs can propagate Ca$^{2+}$ signals within a cell through responses to [Ca$^{2+}$]$\_i$ (33, 34). Hence, excitation-contraction coupling within excitable cells can occur as L-type Ca$^{2+}$ VOCs increase [Ca$^{2+}$]$\_i$, thereby stimulating further Ca$^{2+}$ release via RyRs.

Although Ca$^{2+}$ binding is a potent agonist of RyR channel opening, higher levels actually induce channel closure. IP$_3$Rs absolutely require inositol 1,4,5-trisphosphate (IP$_3$) for activation; however, they also can be considered CICR channels similar to RyRs, since cytosolic Ca$^{2+}$ has a comparable bell-shaped effect on the open probability of the Ca$^{2+}$ channel pore (102). Extracellular agents can trigger [Ca$^{2+}$]$\_i$ release from intracellular stores through activation of specific cell surface receptors and engagement of the signal transduction pathway, which involves activation of phospholipase C (PLC); furthermore, the phospholipase-dependent catalysis of phosphatidylinositol 1,4-bisphosphate liberates IP$_3$, the diffuseable messenger that binds to and activates IP$_3$Rs.

Overall, CICR channels are interrelated by an ability to permeate Ca$^{2+}$, a functional sensitivity to local Ca$^{2+}$ concentrations and by an organization on membranes that facilitates Ca$^{2+}$-mediated communication between like macromolecules (127). The IP$_3$Rs and RyRs share these CICR features as transmembrane ROCs with similar but distinct structural and functional characteristics. Architecturally, vertebrate IP$_3$Rs and RyRs are large proteins forming functional channels as symmetric tetramers. The ion-conducting region of IP$_3$Rs and RyRs share a high sequence homology. Furthermore, the commonalities between these ROCs spread beyond the pores, with conserved mannosyl-transferase, IP$_3$, RyR (MIR), internal RyR and IP$_3$, RyR homology (RIH), as well as RIH-associated domains before the transmembrane regions. MIR domains are a series of ~200 amino acid repeats near the NH$_2$ terminus, also found in yeast o-mannosyl-transferase that do not form discrete IP$_3$-binding sites (72). RII domains are also located near the NH$_2$ terminus, exhibiting sequence and structural homology to the IP$_3$-binding core of IP$_3$Rs (97). Calcium release channels often contain RIH domains coupled with RIH-associated domains, an ~100 amino acid domain preceding the
pore-forming regions (72, 97). RyRs, which are composed of almost double the number of amino acids as IP$_3$Rs, diverge from IP$_3$Rs with the insertion of several NH$_2$-terminal and COOH-terminal pore-intervening SPRY (SP1a and RyR) domains. SPRY domains share low sequence identity and function as protein-interaction domains (121). The conserved architectural features found in IP$_3$Rs and RyRs suggest the presence of a common ancestor for these ROCs in lower order organisms.

The present review focuses on new structural insights on the NH$_2$-terminal regions of IP$_3$Rs and RyRs and the tetrameric arrangement of these regions exhibiting high structural homology in the full-length context. Additionally, we highlight genomic analyses identifying this class of ROCs in unicellular organisms and the implications of these data in understanding the molecular evolution of these fundamental receptors in nature.

Genomic Analyses and Domain Organization of IP$_3$Rs and RyRs

The IP$_3$R genes encode large proteins (~2,700 amino acid residues) that assemble into tetrameric functional channels. The RyR gene encodes an even larger subunit (~5,000 amino acid residues), also forming a tetrameric channel on the membrane. Three isoforms of IP$_3$Rs (IP$_3$R1, IP$_3$R2, and IP$_3$R3) and RyRs (RyR1, RyR2, and RyR3) have been identified in mammalian vertebrates (9, 26, 38, 52, 65, 73, 81, 91, 104, 118). In nonmammalian vertebrates such as birds and fish, two to three IP$_3$R isoforms and two RyR isoforms (RyRN and RyR6) are expressed (92, 120). In mammals, the three IP$_3$R isoforms are ubiquitously expressed and have distinct and overlapping distribution patterns; cells in the central nervous system predominantly or solely express IP$_3$R1, whereas most other cells express more than one type (14, 27, 28, 84, 89, 116, 124, 126, 132). The RyR1 isoform is primarily expressed in skeletal muscle (122, 141), whereas the predominant form of RyR in cardiac muscle is RyR2 (85, 91). RyR2 is also expressed at high levels in Purkinje cells of the cerebellum and the cerebral cortex (37, 62, 86, 115). RyR3 is expressed in hippocampal neurons, thalamus, Purkinje cells, corpus striatum (37, 42, 62), skeletal muscles (75, 90), and smooth muscle (39, 40, 43, 92).

Although RyRs can be activated by Ca$^{2+}$ alone, IP$_3$Rs are classified as a dual-ligand gated channel whose opening is induced by IP$_3$ and Ca$^{2+}$ (7, 35, 49, 50). Additionally, a diverse number of molecules are known as modulators of IP$_3$Rs and RyRs and are largely responsible for distinctions in function. IP$_3$R channel activity can be regulated by divalent cations, ATP, PKA, PKG, CaMKII, Akt, CaM, CaBP1, ERp44, IRBIT, RACK1, 4.1N, Homer, TRPC, and Na$^{+}$-K$^{+}$-ATPase, to name a few (36, 127). Similarly, RyR function is modulated by molecules such as ryanodine, Mg$^{2+}$, ATP, PKA, CaMKII, Ca$_{1,1}$, CaM, calsequestrin, and FKBP12 (65).

Despite vast differences in protein size, vertebrate IP$_3$Rs and RyRs share a similar domain architecture (FIGURE 1A). Both receptors possess pore-forming transmembrane helices at the COOH terminus, contain critical NH$_2$-terminal cytosolic domains that regulate the channel helices, and have large cytoplasmic regulatory domains sandwiched between the NH$_2$ and COOH termini. Recently, the crystal structures of NH$_2$-terminal domains of rat IP$_3$R1 (residues 1–604) and rabbit RyR1 (residues 1–559) were revealed to be strikingly similar despite low sequence identity (111), the topic of detailed discussion below. These functional and structural similarities between vertebrate IP$_3$Rs and RyRs suggest a common ancestry between IP$_3$R and RyRs. In multicellular invertebrates, genes encoding IP$_3$R, itr-1, and RyR, unc-68, have been identified in Caenorhabditis elegans (3, 76, 107). Despite studies in past decades identifying G-protein-coupled receptors and IP$_3$-associated second messenger systems in unicellular eukaryotes (17, 22, 77), only recent genomic analyses have revealed the presence of putative IP$_3$Rs and RyRs in Monosiga brevicollis, Salpingoeca rosetta, and Capsaspora owczarzaki, the closest unicellular relatives of animals (15, 16). Putative IP$_3$R/RyR homologs have also recently been identified in pathogenic unicellular parasites including Trypanosoma brucei, Trypanosoma cruzi, Leishmania major, and Leishmania infantum (97).

The phylogenetic relationship of IP$_3$Rs and RyRs in unicellular and multicellular eukaryotes suggests that the divergence of IP$_3$Rs and RyRs occurred along with or shortly before the emergence of multicellular organisms, since the IP$_3$R/RyR homolog in Trypanosoma and Leishmania branched out before the IP$_3$Rs and RyRs (FIGURE 1B). Hence, these homologs are likely common ancestors to the eukaryotic Ca$^{2+}$ release channels and may possess a rudimentary activation mechanism, the critical features of which are inherited by IP$_3$Rs and RyRs in multicellular animals. For example, the unicellular IP$_3$R and RyR homologs show a high degree of similarity (~40%) in the pore region responsible for ion conductance compared with mouse IP$_3$R1 (residues 2,527–2,596) and mouse RyR1 (residues 4,874–4,942), highlighting the pore region as a key structural feature in intracellular Ca$^{2+}$ channel function. The IP$_3$R/RyR homologs in Trypanosoma and Leishmania are much shorter than RyRs found in multicellular animals, and evaluation of the genomes for the presence of an IP$_3$-binding domain (i.e., based on mouse IP$_3$R1 residues 224–604) confirms a
closer homology to IP₃Rs than RyRs, despite lacking many of the basic residues essential for high-affinity IP₃ binding in vertebrates (10). These data, taken together with the fact that only IP₃R (i.e., not RyR) has been identified in the unicellular *Paramaecium* (61), suggest that RyRs appeared later in evolution. Future high-resolution structural work should provide further insight into the molecular evolution of this class of receptors. At present, detailed structural data exists only for the NH₂-terminal domains of the vertebrate IP₃R and RyRs; nonetheless, the data have provided a wealth of knowledge on the commonalities in mechanistic action between IP₃Rs and RyRs.

**Atomic Resolution Structures of the NH₂-Terminal Domains of IP₃Rs and RyRs**

The NH₂-terminal region of vertebrate IP₃Rs, which resides in the cytosol, contains two functional domains: the suppressor domain (SD; residues 1–223 of IP₃R₁) and the IP₃-binding core (IBC; residues 224–604 of IP₃R₁). The first high-resolution structure determined by X-ray crystallography was the IBC of IP₃R₁ (PDB code 1N4K) (10). The structure of the IBC in complex with IP₃ contains two structurally distinct domains: the β-domain

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**FIGURE 1. Domain organization and genomic analyses of IP₃Rs and RyRs**

**A.** Domain architecture for IP₃R and RyR. The numbers on top of the domain diagram represent the amino acid residues of rat IP₃R₁ and rabbit RyR₁. NTD, NH₂-terminal domain; SD, suppressor domain; IBC, IP₃-binding domain; TMD, transmembrane domain; CTT, COOH-terminal tail.

**B.** Maximum likelihood phylogenetic tree showing the relationship between IP₃R and RyR homologs in uni- and multicellular eukaryotes. The blue and yellow shades separate the sequences of IP₃Rs and RyRs. Bootstrap values of >50 are shown at the nodes. The scale bar represents amino acid substitutions per site. Multiple sequence alignments were made with MAFFT using default parameters (58). After using Gblocks 0.91b (19) at low stringency to remove regions of low confidence and gaps, maximum likelihood analysis was carried out using MEGA 5.0 using default parameters with the WAG substitution model (123) (100 bootstrapped data sets).
The IBC-β (residues 224-436 of IP₃R₁) adopts a β-trefoil fold containing 12 β-strands and two single turn helices, whereas the IBC-α (residues 437-604 of IP₃R₁) adopts an armadillo repeat fold consisting of 8 α-helices (FIGURE 2A). The IBC forms an L-shaped structure with the two domains oriented approximately perpendicular to each other, and the basic amino acids located in the cleft formed by both domains comprising the IP₃ binding site. The crystal structures of the SD have been determined for IP₃R₁ (residues 1–223 of IP₃R₁, PDB code 1XZZ) (11) and IP₃R₃ (residues 1–224 of IP₃R₃, PDB code 3JRR) (21); moreover, the two structures are nearly identical (FIGURE 2B). The SD folds into a hammer-like structure with a 12-β-stranded “head” domain and a helix-turn-helix “arm” domain. Furthermore, the head domain of the SD adopts a similar β-trefoil fold found in the IBC; however, the molecular surfaces of specific

**FIGURE 2. Structural features of the IP₃R-NT region**

A: structure of the IBC in complex with IP₃ at 2.2-Å resolution. Ribbon and surface representations of the IBC-β (light green) and IBC-α (orange) are shown, and the IP₃-coordinating residues are colored red. B: superimposed structures of the IP₃R₁-SD (blue) and IP₃R₃-SD (brown). The acidic pocket in IP₃R₃-SD and the equivalent region in IP₃R₁-SD are highlighted (magenta circle) and represented by electrostatic surface. C: superimposed apo-NT (SD, blue; IBC-β, red; IBC-α, light green) and IP₃-bound NT (gray) structures. The structures were aligned by overlaying the IBC-β. D: the two interfaces between the SD and IBC (colored as in C) domains. The β-interface (top) and α-interface (bottom) are shown. E: a model depicting the IP₃-evoked conformational changes and the coupling between SD and channel gating. The critical loop (including Tyr167 residue) for channel gating is colored red. The IP₃ molecule and transmembrane domains (TMD) are shown as a violet triangle and gray cylinders, respectively.
regions are considerably different between SDs from type I (IP$_3$R1-SD) and type 3 (IP$_3$R3-SD), and this may contribute to isoform-specific IP$_3$ binding affinity (51). Specifically, the IP$_3$R3-SD contains a small acidic pocket centered by the negatively charged Glu39 that is replaced by Gln40 in IP$_3$R1-SD; moreover, the side-chain orientation of Arg53 near the pocket in IP$_3$R3-SD is retracted compared with the orientation of the corresponding residue (Arg54) in IP$_3$R1-SD, which collapses the pocket (FIGURE 2B). It is noteworthy that subtype-specific IP$_3$ binding equilibrium dissociation constants ($K_d$) have been estimated in the range of $10^{-7}$, $10^{-8}$, and $10^{-6}$ M for type 1, 2, and 3 IP$_3$R, respectively (51, 52). Furthermore, modifications to the SD, such as posttranslational phosphorylation and heteromeric protein interactions (e.g., with calmodulin, CaBP1, and RACK1), could potentially influence IP$_3$ binding affinity. Functionally, different sensitivities to IP$_3$ binding, through isoform-specific structural differences as described for IP$_3$R1 and IP$_3$R3, posttranslational modifications, or heteromeric protein-interactions, play a role in shaping the spatial and temporal Ca$^{2+}$ release responses that drive specific cellular processes. Consistent with this notion, dynamically changing IP$_3$ sensitivity has been reported during Ca$^{2+}$ oscillations in live cells (78).

The isolated IBC is the minimal domain required for IP$_3$ binding and shows a very high affinity for IP$_3$ in vitro ($K_d \sim 2.3$ nM). Remarkably, the affinity of the entire NH$_2$-terminal region (NT; residues 1–604 of IP$_3$R1) is reduced by more than one order of magnitude ($K_d \sim 45$ nM), implying that the SD inhibits (or “suppresses”) the IP$_3$ binding (139). Not only is the SD required for suppression of IP$_3$ binding, but it is also needed for IP$_3$-induced allosteric channel gating. A single Tyr167Ala mutation in the SD attenuates IP$_3$-evoked Ca$^{2+}$ release (138). Tyr167 is located on the exposed loop in the head domain of SD and oriented on the opposite face of the SD/IBC interfaces. Although the exact allosteric mechanism of the functional coupling between the SD and channel domain remains elusive, the critical loop in the SD that includes Tyr167 plays an important role in linking IP$_3$ binding to channel gating.

Recently, four atomic-resolution NT structures of IP$_3$R1 have been determined. Lin and colleagues solved two NT structures of rat IP$_3$R1 at 3.8-Å resolution; moreover, they were able to derive one structure in an IP$_3$-free state (i.e., apo) and a second structure in an IP$_3$-bound state (i.e., holo) from a single crystal grown in the presence of IP$_3$ (PDB code 3T8S) (66). Subsequently, the NT structures of rat IP$_3$R1 at higher resolution were separately determined from crystals grown in the absence (3.0 Å, PDB code 3UJ4) and presence (3.6 Å, PDB code 3UJ0) of IP$_3$, respectively (111) (FIGURE 2C). The individual structures of the three domains comprising NT of IP$_3$R1 (i.e., SD, IBC-β, and IBC-α) are remarkably similar to the separately determined SD (PDB code 1XZZ) (11) and IBC (PDB code 1N4K) (10). Nonetheless, these NT structures represent a significant advancement in the understanding of IP$_3$R function by revealing the arrangement of the SD and IBC domains with respect to one another and providing important clues on the bases for tetrameric channel formation. The three domains in NT structure form a triangular structure, and the SD is located on the opposite face of the IP$_3$-binding site, suggesting that the SD suppresses the IP$_3$ binding by an allosteric mechanism (FIGURE 2C). The SD interacts with both the IBC-β and IBC-α, respectively, and forms two interfaces (i.e., β-interface and α-interface). The short β-interface consists predominantly of hydrophobic interactions between Pro49, Phe53, and Phe223 from the SD, and Pro291 and Ala292 from the IBC-β, and is supported by a salt bridge between Lys225 and Asp228 (FIGURE 2D, TOP). The longer α-interface is stabilized by hydrophobic interactions between Val33 and in the SD and a pocket formed by nonpolar Val452, Phe445, Ala449, and Leu476 within IBC-α. Electrostatic interactions between Arg54 and Lys127 from the SD and Asp444 from IBC-α are also involved in forming the α-interface (FIGURE 2D, BOTTOM) (111). The functional importance of residues associated with the α-interface is demonstrated by the Val33Lys mutation, which almost completely abrogates the effects of the SD on IP$_3$ binding and attenuates the maximal open probability of the full-length channel (11, 105).

The most marked conformational change caused by IP$_3$ binding is the significant decrease in the domain orientation angle between IBC-β and IBC-α (FIGURE 2, C AND E). This ligand binding-induced structural change in IBC occurs with the hinge region set between IBC-β and IBC-α as a pivot point, resulting in a narrowing of the IP$_3$-binding cleft. Although the interdomain distance between SD and IBC-β at the β-interface slightly increases, the hydrophobic and electrostatic interactions forming the α-interface are retained after IP$_3$ binding; consequently, the SD rotates toward the IBC by ~9°, and twisting occurs approximately perpendicular to the closure of IBC. Ultimately, this subdomain reorientation within NT causes the movement of the exposed loop in the SD, including Tyr167 (FIGURE 2E).

The first X-ray studies on RyRs elucidated structures of the distal NH$_2$-terminal region (residues 1–205, domain A) for RyR1 (PDB code 3HSM) (1) and RyR2 (PDB code 3IM5) (69), demonstrating that domain A folds into a β-trefoil core consisting

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of 12 β-strands and a single α-helix. Inheritable mutations in RyR1 and RyR2 have been linked with muscle contraction diseases including malignant hyperthermia (MH) (12, 13, 18, 29, 31, 47, 57, 60, 64, 88, 93–95, 103, 106, 134), central core disease (CCD) (2, 30, 54–56, 98, 100, 101, 114, 140), catecholaminergic polymorphic ventricular tachycardia (CPVT) (25, 32, 41, 45, 46, 48, 59, 63, 67, 74, 87, 96, 128, 130, 135), and arrhythmogenic right ventricular dysplasia (ARVD) (23, 64, 83). These initial structures showed that a high frequency of disease-associated mutations for RyR1 (MH and CCD) and RyR2 (CPVT and ARVD) cluster in and around a loop connecting β8 and β9 [the hot spot (HS) loop] (1, 69). X-ray crystallography and nuclear magnetic resonance (NMR) experiments do not show any significant structural differences between the mutants and wild-type proteins, and biophysical analyses suggest no significant changes in stability (1, 69), unlike numerous other protein conformational disorders. Therefore, RyR mutations may perturb quaternary RyR interactions involved in tetrameric channel formation or interactions with modulatory proteins (119, 125).

More recent X-ray crystallography has revealed the structure of the first 559 residues of RyR1 (PDB code 2XOA), providing new insights into the effect of disease-associated mutations on RyR structure and function (129). This larger NH2-terminal construct encompasses three domains (i.e., A, B, and C). Although both domains A and B are comprised of β-trefoil cores (1, 69), domain C folds into a five-helix bundle (129). The domains interact with each other primarily via hydrophilic interactions, and 56 different disease-associated mutations from RyR1 and RyR2 can be mapped onto this larger structure (FIGURE 3A) (129). Most of these mutations are found at the intra-molecular interfaces between the three domains or inter-molecular contacts with neighboring ABC subunits in the tetrameric channel, as modeled by cryo-EM docking studies (see below). Interestingly, 19 of the 56 mutations (~33%) are modeled at the interface between domain A and B on different subunits (129). The HS loop in domain A (1, 69) and two flexible loops from domain B are important components of this inter-subunit interface (129). A second interface concentrated with mutations lies between domains A and C of the same subunit. This A–C intra-molecular interface is stabilized by two salt bridges: Arg45 from domain A interacts with Asp447 in domain C, whereas Asp61 and Ghu40 from domain A form ionic bonds with Arg402 in domain C (FIGURE 3B) (129). Gel filtration and thermal melt experiments on the disease-associated Arg402Gly mutation show only minimal perturbations to the overall fold and stability of the structure (129). Lastly, several point mutations are concentrated at the interface between domain A and electron-dense columns that extend toward the transmembrane domains (129). In addition to point substitutions within ABC, deletion of exon 3 encoding a portion of domain A in RyR2 was linked to CPVT and other heart abnormalities in 16 members from two separate families (8, 79). Remarkably, the structural integrity of RyR2-A is rescued via the insertion of a flexible loop into the β-trefoil domain affected by the deletion resulting in enhanced thermal stability (68). Hence, CPVT is likely manifested by a disruption of interfaces with other domains for individuals carrying this exon 3 deletion (68). The distribution of mutations on inter- and intra-molecular interfaces suggests that gating of the channel is allosterically coupled to the movement of individual domains relative to each other and on different subunits.

The three well folded and isolated domains of IP3R1-NT (SD, IBC-β, and IBC-α) can be superimposed on the corresponding domains of RyR1-ABC (A, B, and C) with a relatively low root mean square deviation in the backbone atoms (i.e., <3 Å). Furthermore, the relative orientation of the three domains is nearly identical when the full NT and ABC structures of IP3R1 and RyR1 are superimposed (FIGURE 3B). The NT and ABC sequence identity and similarity are low (17% and 37%, respectively); however, the sequence and structure of the functionally important loop regions in the two receptors are conserved. In particular, the backbone and side-chain conformation of the critical loop in SD that is essential for channel gating of IP3Rs is structurally homologous to the HS loop of RyR (FIGURE 3B, red box). The structural conservation between IP3R and RyR is also readily apparent at the interfaces; the bidentate salt bridges stabilizing the α-interface of IP3R (Arg54/Lys127 and Asp444) are preserved in the A–C interface of RyR1 (Glu40/Asp61 and Arg402), although with a reversal of charges (FIGURE 3B, black boxes). RyR1 exhibits a salt bridge at the A–C interface (Arg45 and Asp447) where mutational disruption results in disease and is not conserved in the α-interface of IP3R. The exceptionally high structural conservation observed for the cytosolic NH2-terminal regions of IP3R1 and RyR1 extends to the quaternary arrangement of these domains on the membrane, and recent electron microscopy work has provided further insight into the structure-function relationships of this class of ROCs.

Cryo-Electron Microscopy of Tetrameric IP3Rs and RyRs

Several groups have successfully employed cryo-electron microscopy (EM) to determine the quaternary arrangements of these proteins at nanometer
resolution (70, 71, 108). The IP₃R forms a ~1.2 MDa homotetrameric ion channel, and previous studies have suggested that multiple ligand-bound conformations exist in both open and closed states (20, 117). The inherent structural heterogeneity of the full-length IP₃R is underscored by the previous EM studies yielding inconsistent models determined at nominal resolution of 15–34 Å (24, 43, 44, 53, 110, 112). Recently, Ludtke et al. determined the cryo-EM structure of IP₃R₁ isolated from the rat cerebellum at 9.5-Å resolution [EM Data Bank (EMDB) code 5278] by achieving higher image contrast through improved sample preparation (71). The tetrameric structure of IP₃R₁ revealed a mushroom-shaped overall architecture with a fourfold symmetry axis along a central plug (FIGURE 4A). The cytoplasmic region is located above the transmembrane region with several large openings or “windows” between two regions (FIGURE 4B). The well defined transmembrane region shows that IP₃R₁ contains many features of the ion-conduction pore assembly of K⁺ channels (FIGURE 4C) (71). In contrast, the cytoplasmic region consists of a relatively rigid exterior surface with a more structurally variable and flexible interior, possibly due to multiple conformations of IP₃R₁ in the closed state.

The assembled RyR is the largest known membrane protein to date and forms a ~2.2 MDa tetramer (1). The cryo-EM structure of RyR₁ isolated from the rabbit skeletal muscle was first revealed in its closed state at 2.4-nm resolution (99, 113) and further refined to ~10-Å resolution (EMDB codes 1275 and 5014) (70, 109). The tetrameric structure of RyR₁ has an enormous cytoplasmic region that rests on top of a smaller transmembrane region with a fourfold symmetry axis along the ion conduction pore (FIGURE 4, D AND E). Although the cytoplasmic region of RyR₁ does not contain a central plug or the perforations found in the IP₃R₁ structure, the channel region resembles the ion-conduction pores of K⁺ channels (FIGURE 4F), as observed for IP₃R₁. Furthermore, Samsó et al. used EGTA or an agonist, PCB 95, to selectively characterize the open and closed states of RyR₁ bound to FKBP12 (EMDB codes 1607 and 1606, respectively) (108), revealing that a change in channel pore size is accompanied by the twisting movement of the transmembrane region as well as considerable conformational changes throughout the entire cytoplasmic region.

Together with the high-resolution crystal structures of the NH₂-terminal fragments (66, 111, 129), comparison of the cryo-EM structures of full-length IP₃R and RyR has revealed the architectural and functional similarities shared between these ROCs. Docking the crystal structures of IP₃R₁-NT and RyR₁-ABC into their full-length structures shows that their NH₂ termini are located near the top of the cytoplasmic regions (FIGURE 4, B AND E). The IP₃R₁ and RyR₁ NH₂-terminal segments form remarkably homologous tetrameric rings around the fourfold symmetry axis, with the disease-associated HS loop of RyR₁ (residues 152–167) and the equivalent loop of IP₃R₁ (residues 165–180) involved in intersubunit interactions (FIGURE 4, A AND D). Hence, these observations indicate that the NH₂-terminal regions of RyRs and IP₃Rs play a critical role in the allosteric modulation of ion channel conductance through a modification of quaternary arrangements.

FIGURE 3. Disease-mutation localization and structural homology between RyR-ABC and IP₃R-NT

A: the structure of the ABC domain from RyR₁ at 2.5-Å resolution. Domains A, B, and C are shown with disease-associated mutations highlighted in red. The HS loop is shown in orange, whereas mutations critical for salt-bridge formation are marked with asterisks.

B: comparison of the NH₂-terminal structures of apo-IP₃R₁ and RyR₁ (yellow). The structures were aligned by overlaying IBC-β and domain B. The critical gating loop in IP₃R₁ and the HS loop in RyR₁ are highlighted with a red rectangle, and the conserved residues are represented as sticks in at top right (IP₃R₁, black lettering; RyR₁, red lettering). The α-interface of IP₃R₁ and the corresponding interface in RyR₁ are boxed with a black rectangle, and the preserved salt bridges are depicted in at bottom right.
Concluding Remarks

In multicellular eukaryotes, the universality of Ca²⁺ signaling has evolved with the versatility of the Ca²⁺ signaling toolkit in which IP₃R and RyR are pivotal players (6). The conservation of these Ca²⁺ channels from unicellular eukaryotes to humans suggests that Ca²⁺ release from intracellular storage organelles through Ca²⁺ channels is a defining characteristic among eukaryotes. Structural

**FIGURE 4. Structures of IP₃R and RyR tetramers**

Cryo-EM structures of IP₃R1 (A–C) (EMDB code 5278) and RyR1 (D–F) (EMDB code 1275) in the closed state are shown from top (A and D) and side (B, C, E, F) views. Docked crystal structures of the NH₂-terminal segments of IP₃R1 (PDB code 3UJ0) and RyR1 (PDB code 2XOA) are shown as ribbon representation with SD and A domains in blue, IBC-β and B domains in green, and IBC-α and C domains in red. The HS loops in RyR1-A and IP₃R1-SD are indicated by magenta loops. In C and E, cryo-EM structures are sliced through the fourfold symmetry axis with the cutting plane in dark gray. The luminal side of the ion conduction pore is indicated by arrowheads. The central plug (P) and windows (W) of IP₃R are shown by arrows. The membrane bilayer is depicted as dotted lines. The scale bar applies to all panels in both dimensions.
and genomic analyses have highlighted the critical features of these IP₃Rs and RyRs, which include the ∼600 structurally conserved distal NH₂-terminal residues, TMD pore-forming region, and quaternary assembly of these proteins. Despite the vast differences in molecular size between these ROCs, the fundamental nature of these elements to Ca²⁺ release channel function is corroborated by the evolutionary preservation of these characteristics from low- to high-order eukaryotic Ca²⁺ channels. Additionally, direct evidence for the importance of these elements is shown by experiments that exchange the SD of IP₃R1 with domain A of RyR1; these RyR1A-IP₃R1 chimeras maintain the ability to assemble into a tetrameric channel with similar IP₃ sensitivity and magnitude of Ca²⁺ release from ER stores as wild-type IP₃R1 in live cells (111). Furthermore, replacing the residues downstream of IP₃R1 TMD1 with the aligned TMDs from RyR1 produces a chimera capable of releasing ER Ca²⁺ in response to IP₃ binding with a channel pore that can be blocked by ryanodine, as per wild-type RyRs (111). Taken together, these data indicate a co-evolution of RyRs and IP₃Rs in eukaryotes and a functional interchangeability of key structural domains between these ROCs.

Marked advances in understanding the relationship between the structure and function of Ca²⁺ release ROCs have been made in the past decade highlighting the basis for signal transduction initiation by the NH₂-terminal IP₃R region along with a remarkable conservation in the overall structural architecture in the corresponding region of RyR, despite an absence of the IP₃ ligand binding property. Furthermore, reconstructed images of the tetrameric molecular arrangement of both receptors reveal a striking similarity in channel height in the membrane-anchored conformation despite the approximately twofold difference in polypeptide chain length. These important discoveries have brought new questions regarding the signaling and regulatory mechanisms of these Ca²⁺ ROCs to the forefront. First, how does Ca²⁺ binding regulate both IP₃R and RyR function in a bell-shaped manner? Second, how is IP₃R and RyR function distinctly modulated by the host of cellular signals such phosphorylation, ATP binding, and smaller proteins (i.e., CaBP1), to name a few? Third, what are the structures of the channel pores and how do they change in response to these various cellular stimuli? Future structural and functional studies of IP₃R/RyR homologs in lower eukaryotes are also needed to enhance our understanding of the evolutionary significance of these Ca²⁺ toolkit components and to fully appreciate the rudimentary mechanisms of channel activation and modulation.

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