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Linking Structure to Function: Recent Lessons from Inositol 1,4,5-trisphosphate Receptor Mutagenesis

David I. Yule[*], **Matthew J. Betzenhauser**[**], and **Suresh K. Joseph**[#]

[*]Department of Pharmacology and Physiology, University of Rochester, Rochester, NY

[#]Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA

[**]Department of Physiology & Cellular Biophysics, Columbia University Medical School, New York, NY

Abstract

Great insight has been gained into the structure and function of the Inositol 1,4,5 trisphosphate receptor ($InsP_3R$) by studies employing mutagenesis of the cDNA encoding the receptor. Notably, early studies using this approach defined the key constituents required for $InsP_3$ binding in the N-terminus and the membrane spanning regions in the C-terminal domain responsible for channel formation, targeting and function. In this article we evaluate recent studies which have used a similar approach to investigate key residues underlying the *in vivo* modulation by select regulatory factors. In addition, we review studies defining the structural requirements in the channel domain which comprise the conduction pathway and are suggested to be involved in the gating of the channel.

In the 20 years following the cloning of the type-1 inositol 1,4,5-trisphosphate receptor ($InsP_3R$-1) [1] analysis of the predicted amino acid sequence has proceeded with studies of mutant $InsP_3R$ proteins in an continuing effort to link structure to function. Indeed, the initial sequence analysis of the $InsP_3R$-1 noted several salient features of the protein; a highly positively charged region in the N-terminus, potentially constituting requirements for the putative $InsP_3$ binding region; two Protein Kinase A (PKA) consensus phosphorylation sites in a predicted central cytoplasmic domain and a hydrophobic region in the C-terminus, indicating a putative transmembrane domain. Strong evidence for the latter prediction was that a deletion mutant lacking the C-terminal 534 amino acid residues was, in contrast to the membrane associated native protein, largely soluble [1]. Additional truncation mutations proved invaluable in assigning function to the N- and C- terminal regions of the protein. In particular, deletion of predicted transmembrane domains in the C-terminus resulted in a soluble, monomeric protein confirming that the transmembrane domains were involved in tetrameric assembly and endoplasmic reticulum localization [2]. This study also demonstrated that expression of the N-terminal 788 residues of the protein retained $InsP_3$ binding while further truncation to residue 519 eliminated binding, indicating the boundary for the $InsP_3$ binding domain in the N-terminus of the protein [2].

These initial findings, establishing the location of the binding site and channel have subsequently been greatly extended. Notably, using an extensive collection of glutathione S-transferase (GST) N-terminal receptor constructs and exhaustive mutagenesis, Mikoshiba and colleagues defined the binding "core" of the receptor between residues 223-608. Within this domain, the key positively charged amino acids, which coordinate the negative phosphate groups of $InsP_3$ to mediate high-affinity specific binding were also elucidated [3, 4]. Notably, 9 of the 12 Arginine or Lysine residues identified in these studies were later confirmed to coordinate the binding of $InsP_3$ in a crystal structure of the $InsP_3R$ binding core with $InsP_3$ [5]. Cloning of two further family members revealed that the proteins shared high sequence similarity at the N- and C-termini coupled by a substantial intervening segment with far greater sequence diversity. This region, since termed the regulatory and coupling domain, harbors putative consensus motifs for regulation by a dizzying array of potential modulatory input, including phosphorylation, by cytoplasmic factors such as ATP and by protein binding partners (For review see [6-8]). By virtue of harboring differing subtype specific motifs, the regulatory and coupling domain has been suggested to contribute to the selective regulation of Ca^{2+} release through individual $InsP_3R$ family members.

Despite a wealth of information regarding some aspects of the molecular requirements of $InsP_3R$ function, as noted for example $InsP_3$ binding, there is a relative lack of data addressing the determinants of other features of $InsP_3R$ structure and function. In particular, the residues in the C-terminal channel domain contributing to the conduction pathway, to the gating of the channel and those responsible for coupling binding in the N-terminus to opening of the pore are not established. Also neglected are studies unequivocally establishing that *putative* structural motifs are indeed directly responsible for the *in vivo* modulation of $InsP_3R$ activity. This latter issue, when investigated, has provided some surprising results. In this article, we review studies which have primarily employed $InsP_3R$ mutagenesis to gain insight into some of these unresolved issues.

The $InsP_3R$ modulatory/coupling domain-phosphorylation of $InsP_3R$-1 by PKA

The presence of PKA consensus phosphorylation sites noted in the original reports, were consistent with the observation that the $InsP_3$ binding protein expressed in cerebellar purkinje neurons was a known prominent substrate for PKA [9]. Indeed, in retrospect, the $InsP_3R$-1 was known to be a phosphoprotein prior to its function as Ca^{2+} release channel being established having been identified in an early proteomic screen of brain phosphoproteins [10, 11]. In general, confirmation that a putative regulatory site has relevance for modulating the activity of the protein *in vivo* is a multi step process. In the case of a phosphorylation event, the protein must be shown to be phosphorylated and this observation correlated with an effect on protein function. Ideally, these two events should then be conclusively linked by abrogating the effect on activity by mutation of the phosphor-acceptor amino acid. In terms of PKA phosphorylation, early experiments physically demonstrated that $InsP_3R$-1 was stoichiometrically phosphorylated at two putative phosphorylation sites (S1588 and S1755 in the mouse protein). These residues are present in the canonical PKA substrate motif (RRXS/T; R=basic residue) [12, 13] (Figure 1A/B). Moreover, it was suggested that the major peripherally expressed splice variant of the $InsP_3R$-1, the so-called short form ($S2^-$ $InsP_3R$-1), in which 40 amino acids (Figure 1B; 1692-1732) are excised from the protein between the two phosphorylation sites, was more readily phosphorylated *in vitro* at S1588 than the predominately neuronal $S2^+$ $InsP_3R$ harboring the 40 amino acid insert which was phosphorylated at both sites [12].

Numerous subsequent studies addressed the functional effects of PKA phosphorylation and resulted in contradictory findings; both potentiated and inhibitory effects on $InsP_3R$-1 activity where reported for the $S2^+$ $InsP_3R$-1, while mostly inhibitory effects were reported in experiments were the $S2^-$ $InsP_3R$-1 was presumed to be expressed [14-18]. These studies highlight the general problem of linking a regulatory event to the functional consequence; the activity of the $InsP_3R$ was measured indirectly as a net Ca^{2+} flux and secondly, mutagenesis of the putative site was not performed to unequivocally prove that modification was linked to the change in activity. Obviously both these issues were not trivial given the contemporary tools available, in particular, the lack of a suitable $InsP_3R$ *null* expression system to functionally test the effect of mutations in the receptor. A major advance to the field in this respect has undoubtedly been the creation of the DT40 3KO cell line [19-21], a unique, genetically tractable tool which has allowed unambiguous measurement of $InsP_3R$ function on a *null* background.

Even given this substantial biochemical and functional data, mutational analyses have provided unexpected insights into the effects of phosphorylation on $InsP_3R$-1. A common approach has been to express and compare WT and mutant $InsP_3R$ in the DT40-3KO background. In particular these studies have unambiguously assigned the effects of phosphorylation to particular amino acids [22]. Analyses of alanine substitutions at S1589 and S1755 in the rat receptor established that, despite both sites being phosphorylated *in vitro*, phosphorylation of S1755 was necessary and sufficient for markedly increasing the activity of the neuronal $S2^+$ splice variant. In contrast, phosphorylation of either S1589 or S1755 could augment the activity of the peripheral $S2^-$ isoform. Further insights into the mechanisms by which PKA enhances $InsP_3R$-1 activity were obtained by using "phosphomimetic" glutamate substitutions at S1589 and S1755 [23]. The utility of these mutations is that any effects on activity reflect the inherent properties of the channel and are therefore independent of any cell type specific events such as targeting of PKA through anchoring proteins [24]. The glutamate substitutions exhibited increased sensitivity following agonist stimulation and direct activation by $InsP_3$ uncaging. Once again, the S1755E mutation was sufficient for this effect in the $S2^+$ isoform (S1589E retained activity similar to WT) and both S1589E and S1755E were required for the maximal increase in sensitivity in the context of the $S2^-$ splice variant.

Another unique attribute of the DT40-3KO cell is that direct electrophysiological measurements of $InsP_3R$ activity following expression of defined homotetrameric channels can be made in native membranes. These measurements can be performed in the "on nucleus" configuration pioneered by Foskett and colleagues [25-29] or by whole-cell measurements of channels expressed in the plasma membrane as detailed by Taylor and co-workers [30-32]. Mechanistic information regarding the enhancing effects of phosphorylation was provided by single channel measurements of plasma membrane $InsP_3R$-1 in stable DT40 cell lines. This technique, allows for direct channel measurements from a small numbers of channels in the plasma membrane (typically less than 4) while in the whole-cell patch clamp configuration. Similar to Ca^{2+} imaging experiments and lipid bilayer channels measurements [16], the primary effect of the phosphomimetic mutations, like actual phosphorylation, was to cause an increase in P_o at sub-saturating [$InsP_3$]. Interestingly, there was no apparent effect on $InsP_3$ binding or the biphasic effects of Ca^{2+} on the receptor. There were however, clear effects on the kinetics of channel opening and closing such that the phosphorylated channel predominately gated in a "bursting mode" characterized by extended periods of rapid channel openings and closings [33].

The $InsP_3R$ modulatory/coupling domain-phosphorylation of $InsP_3R$-1 by PKG

The identical PKA-phosphorylation sites are also potential targets of cGMP-dependent Protein Kinase (PKG) [34-36]. Despite phosphorylation of S1755 being theoretically a preferred site of PKG due to the presence of an upstream aromatic residue (F1759), S1588 has been reported to be the predominant target of PKG in $S2^+$ $InsP_3R$-1 [36]. Again, there is little consensus in the literature as to the site responsible for the functional effects of PKG phosphorylation. Interestingly a similar mutational analysis indicated that the splicing of the $InsP_3R$-1 also appeared to determine the susceptibility of phosphorylation by PKG. Activating PKG resulted in augmented Ca^{2+} signals in $S2^+$ $InsP_3R$-1 expressing cells, an effect attributed to phosphorylation of S1755. Surprisingly, PKG had no effect in cells expressing $S2^-$ $InsP_3R$-1 [23]. The parsimonious explanation for these data, given that phosphate incorporation at either site in the $S2^-$ $InsP_3R$-1 (following PKA activation) results in enhanced activity, is that the particular conformation of the $S2^-$ $InsP_3R$ renders the protein a poor substrate for PKG. These data also indicate that the reported functional effects of PKG activation in tissues such as smooth muscle and megakaryocytes [37] which express $S2^-$ $InsP_3R$-1 are likely to be the consequence of phosphorylation of other $InsP_3R$ family members co-expressed. Alternatively, these effects could be mediated indirectly via an accessory protein such as $InsP_3R$-associated cGMP kinase substrate (IRAG) [38].

The $InsP_3R$ modulatory/coupling domain- PKA phosphorylation of $InsP_3R$-2 and $InsP_3R$-3

Functional effects of PKA phosphorylation have also been attributed to phosphorylation of $InsP_3R$-2 and $InsP_3R$-3 [18, 39-42]. It should be noted however that neither receptor is stoichiometrically phosphorylated following PKA activation [18, 43]. For example, in AR4-2J pancreatoma cells, parotid acinar cells and hepatocytes expressing a predominance of $InsP_3R$-2, an increase in receptor activity has been demonstrated following PKA stimulation [18, 44]. In contrast, in cells expressing a majority of $InsP_3R$-3, such as pancreatic acinar cells and Rin M5F insulinoma cells, both an inhibitory and enhancing effect have been reported [18, 41, 42]. It should be noted that without exception, all these studies have again relied on indirect measurements of $InsP_3R$ activity, further confounded by the fact that all the cells studied express multiple $InsP_3R$ family members to varying extents. Moreover, although PKA consensus motifs are conserved in $InsP_3R$-1 of all species with genomes encoding multiple $InsP_3R$ proteins, these consensus sites are absent in $InsP_3R$-2 and $InsP_3R$-3 protein (Figure 1C and D). Here the problem of conclusive linkage between the site responsible for cause and effect has lagged behind the determination of actual sites phosphorylated in the individual receptors.

The $InsP_3R$ modulatory/coupling domain- PKA phosphorylation of $InsP_3R$-2

Scores of serine and threonine residues are present in minimal PKA consensus sequences (RXXS/T or RRXS/T) within the cytosolic domains of $InsP_3R$-2 making a strictly mutagenesis approach somewhat unattractive. To circumvent this issue, a subcloning approach was taken to identify phosphorylation sites in $InsP_3R$-2 [45]. This strategy was based on the fact that limited trypsin digestion of the $InsP_3R$-1 or $InsP_3R$-3 attacks solvent exposed sites and results in 5 ($InsP_3R$-1) or 4 ($InsP_3R$-3) globular domains [3, 43, 46]. In a similar manner to maintain tertiary structure, the sub-clones were designed around 5 predicted tryptic digest sites in $InsP_3R$-2. Only fragments 3 and 5, corresponding to residues 920-1583 and 1884-2701 were phosphorylated *in vitro*. Mutation of S937A (among 5 serine residues) eliminated phosphorylation in fragment 3 while mutation of S2633A abrogated the

effect in fragment 5, identifying these residues as potential PKA phosphorylation sites in $InsP_3R$-2. S2633 is conserved in all $InsP_3R$ subtypes and has in fact been demonstrated to be phosphorylated by Akt kinase *in vivo* [47, 48]. Because mutation of established PKA sites in both $InsP_3R$-1 and $InsP_3R$-3 abolished all the effects of PKA activation, S2633 is unlikely to represent a PKA phosphorylation site in the intact protein. In contrast, S937 is unique to the $InsP_3R$-2 (Figure 1C) and is conserved in all $InsP_3R$-2 genes in the NCBI database. Of interest, S937 was also independently identified as a phosphorylated residue in a proteomic screen of liver phosphoproteins [49]. Subsequent experiments demonstrated that an antibody raised against phosphorylated S937 recognized a protein product of appropriate size in forskolin treated COS-7 cells expressing $InsP_3R$-2 but not S937A $InsP_3R$-2 and importantly, native receptor in forskolin treated parotid acinar cells. Finally, PKA activation resulted in enhanced Ca^{2+} signals and single $InsP_3R$-2 channel activity in DT40-3KO cells expressing $InsP_3R$-2 but the effect was absent in S937A $InsP_3R$-2 expressing cells [45]. These experiments, taking a deliberate methodological approach establish that S937 is a *bone fide*, functionally relevant phosphorylation site in $InsP_3R$-2. Further, work is obviously necessary to establish the physiological role of this regulation in cells which express predominately $InsP_3R$-2.

The $InsP_3R$ modulatory/coupling domain- PKA phosphorylation of $InsP_3R$-3

Wojcikiewicz and colleagues have reported that of 33 serines and 18 threonine residues present in minimal PKA consensus motifs of $InsP_3R$-3, only S–A substitutions in three sites (S916, S934, S1832) reduced phosphorylation of $InsP_3R$-3 *in vivo* (Figure 1D) [43]. These sites appear to represent the sole sites in the intact $InsP_3R$-3 as a construct harboring S-A mutations in each of the three serine residues is not phosphorylated following PKA activation. Interestingly, while S934 in particular was phosphorylated prominently in DT40-3KO cells stably expressing $InsP_3R$-3, no effect on Ca^{2+} release stimulated by activation of endogenous DT40 cell phospholipase C-coupled receptors was observed in populations of cells [50]. These data again highlight that the physical demonstration of a phosphorylation event does not necessarily correlate with effects on Ca^{2+} release. Further experiments directly measuring channel activity are necessary to rule out that PKA-phosphorylation of $InsP_3R$-3 results in subtle effects on channel activity not evident in population [Ca^{2+}] measurements. An alternative is that phosphorylation of $InsP_3R$-3 has consequences other than directly effecting Ca^{2+} flux for example, by influencing the complement of interacting proteins/factors and thus altering function as a signaling integrator and adaptor protein.

The $InsP_3R$ modulatory/coupling domain-regulation by adenine nucleotides

A further example where mutagenesis of “established” regulatory sites has yielded unexpected results is the modulation of receptor activity by adenine nucleotides. This form of modulation may be important in a variety of physiological and pathological situations by providing direct coupling between the metabolic status of the cell and Ca^{2+} release. ATP was initially demonstrated to increase Ca^{2+} flux [51] and the $InsP_3$-gated activity of channels from smooth muscle [52]. Subsequently, it was established that 8-azido-α^{32}P-labelled ATP bound stoichiometrically to the purified cerebellar $InsP_3R$ at a single binding site [53] and was a potent modulator of $InsP_3$-induced calcium release and channel activity via the reconstituted receptor [53, 54]. A number of studies documented that sub-millimolar concentrations of ATP (and other adenine nucleotides) increase the activity of the channel, while higher concentrations appear to inhibit [55, 56]. ATP modulates all $InsP_3R$ family members, albeit with $InsP_3R$ specific characteristics [57-59]. For example, in Ca^{2+} release assays, where Ca^{2+} is clamped to ~ basal cytosolic levels, ATP appears to modulate Ca^{2+} release through $InsP_3R$-1 and $InsP_3R$-3 at all [$InsP_3$] while $InsP_3R$-2 is only subject to

modulation at sub-maximal [$InsP_3$] [59, 60]. In isolation, the individual receptors also differ in their affinities for ATP modulation, with $InsP_3R$-2 having a ~10 fold higher sensitivity than $InsP_3R$-3 and $InsP_3R$-1 exhibiting intermediate affinity [55, 57-60]. Consistent with these data, native cells expressing a majority of $InsP_3R$-2 are more sensitive to ATP modulation than cells expressing predominately $InsP_3R$-3 [61]. Mechanistically, the inhibitory effect is thought to occur as high [ATP] inhibit $InsP_3$ binding to the receptor [55, 56] while the increase in activity occurs as ATP allosterically modulates the Ca^{2+} co-agonist sensitivity of the $InsP_3R$ [27, 28]. The effects of ATP do not require hydrolysis [54-56], but are thought to be mediated by ATP binding to specific sites in the regulatory domains of the receptors [6, 25, 62].

It has been proposed, and widely accepted that ATP binds to glycine rich regions containing the sequence G-X-G-X-X-G (Figure 1). This sequence is reminiscent of Walker type A motifs common in many ATPase and GTPases [6, 25, 62, 63]. The primary sequence of $InsP_3R$ family members contains a number of such motifs (Figure 1A-D). A region termed the ATPB site is conserved in all three family members (aa 2016-2021 in $InsP_3R$-1), the so-called ATPA site is unique to the $InsP_3R$-1 (aa 1773-1780; Figure 1 A-D) and a further site termed ATPC is introduced by excision of the S2 splice site forming $S2^-$ $InsP_3R$-1 (aa 1574-1765; Figure 1B). It should be noted that it has been demonstrated quite convincingly that these sites in isolation bind ATP. For example, GST fusion proteins harboring the particular sites have been shown to bind fluorescent TNP-ATP or α^{32}P-ATP specifically [59, 64, 65]. Furthermore, mutation of the binding sites by a G-A substitution in the putative ATP binding pocket eliminates binding in this context [59, 65]. Perhaps the most compelling evidence for the relevance of these sites are data which show that 8-azido α^{32}P-ATP can be cross-linked to recombinant $InsP_3R$ which when subjected to controlled proteolysis is found in fragments which are predicted to contain the glycine rich motifs [46]. Specifically, $InsP_3R$-1 was shown to have two binding sites in fragments containing ATPA and ATPB (Figure 1A), while $InsP_3R$-3 bound one ATP molecule in a fragment predicted to harbor ATPB (Figure 1D) [46]. No mutagenesis was carried out in these studies and thus it is formally possible that sites other than GXGXXG motifs are present in these fragments. The presence of two putative binding sites in $InsP_3R$-1 has also been used to explain the higher functional sensitivity of the $InsP_3R$-1 to ATP when compared with $InsP_3R$-3. This explanation is consistent with the reduced functional sensitivity to ATP modulation of the *Opisthotonos (opt)* mutation in $InsP_3R$-1 which results in a deletion of 107 amino acids including the ATPA site [66]. This mutation does however result in deletion of an extended stretch of amino acids (removal of G1732-Q1839) and thus a possibility exists that disruption of overall $InsP_3R$ structure in *opt* alters ATP binding outside the immediate deleted region.

Role of the ATPC site in $S2^-$ $InsP_3R$-1

Despite the weight of persuasive evidence that these domains bind ATP and that the presence of these sites correlates with regulation of function, a firm causal link is missing in the absence of mutagenesis in the full length receptors. Efforts to address this issue have been made by mutating all recognized ATP binding motifs in each of the $InsP_3R$ family members. The primary motivation for this work was to unequivocally establish that the effects of ATP binding at particular sites indeed leads to regulation of $InsP_3R$ function. Since the creation of the ATPC site in $S2^-$ $InsP_3R$-1 is the only obvious structural difference between the $S2^+$ and $S2^-$ splice variants this alteration could potentially contribute to the previously noted splice specific regulation of these receptors [22, 66, 67]. To confirm nucleotide binding to this putative site, TNP-ATP was shown to specifically interact with GST fusion proteins containing the ATPC site. Importantly, this binding was eliminated by substitution of the central glycine residue for alanine in the binding motif (G1690A). In cells

expressing $S2^-$ $InsP_3R$-1, Ca^{2+} release was shown to be markedly increased by ATP, however the mutation of ATPC in the full length $S2^-$ $InsP_3R$-1 (ΔATPC S2- $InsP_3R$-1) did not alter this modulation. Instead, the mutation eliminated PKA phosphorylation and the resultant potentiated Ca^{2+} release [65]. The conclusion from these data was two-fold; that ATP binding to ATPC appears to be necessary for a conformation of the $S2^-$ $InsP_3R$-1 which is susceptible to PKA phosphorylation and secondly that ATP regulation of Ca^{2+} release *per se* occurs at an alternative site, presumably the ATPA or ATPB also present in $InsP_3R$-1.

Role of the ATPB site in $InsP_3R$-2 and $InsP_3R$-3

Experiments addressed whether the single conserved ATPB site in $InsP_3R$-2 and $InsP_3R$-3 is responsible for ATP regulation of Ca^{2+} release in these receptors [59]. To ensure disruption of the ATP binding pocket each glycine residue in $InsP_3R$-2 ATPB was replaced with alanine (Figure 1C; G1969A, G1971A and G1974A; termed ΔATPB $InsP_3R$-2). This construct eliminated ATP regulation of $InsP_3$-induced Ca^{2+} release. In addition, both the oscillation frequency and amplitude of Ca^{2+} signals following B cell receptor (BcR) activation were reduced in cells expressing ΔATPB $InsP_3R$-2 when compared to WT $InsP_3R$-2. These data confirm that the ATPB site in the context of $InsP_3R$-2 is solely responsible for mediating the effects of ATP binding and that nucleotide binding has functional consequences after stimulation of an endogenous signaling pathway. In contrast, in the $InsP_3R$-3 a similar mutation in ATPB (Figure 1D; G1920A, G1922A, G1925A; termed ΔATPB $InsP_3R$-3) regulation of $InsP_3$-induced Ca^{2+} release and BcR-induced Ca^{2+} signaling were unchanged [59]. These data indicate that despite evidence of ATP binding in a region that contains the ATPB site that the site itself is functionally irrelevant for regulation of Ca^{2+} release via $InsP_3R$-3. This finding although surprising, nevertheless appears in hindsight, consistent with $InsP_3R$-2 and $InsP_3R$-3 having markedly different characteristics of ATP modulation [58, 59].

Role of the ATPA and ATPB site in $S2^+$ $InsP_3R$-1

In a similar fashion to $InsP_3R$-3, mutation of the $InsP_3R$-1 to create ΔATPB $InsP_3R$-1 (G2016A, G2018A, G2021A $InsP_3R$-1) resulted in no obvious alteration in the sensitivity of Ca^{2+} release to ATP [60]. Given the presumed higher affinity of the ATPA site potentially masking any role of ATPB these data were not unexpected. Remarkably, however when $S2^+$ $InsP_3R$-1 was constructed devoid of any known ATP binding sites (mutation of G1773, G1775A,G1777A, G1780A in ATPA and G2016A, G2018A, G2021A in ATPB; $S2^+$ $InsP_3R$-1 ΔATPA/B) the modulation of Ca^{2+} release by ATP was unaltered. Indeed, at the single channel level, monitored in the “on-nucleus patch” configuration, modulation of Po by ATP, the single channel conductance and the predominant mode of gating of the mutant channel were identical to native $S2^+$ $InsP_3R$-1 [60]. The inescapable conclusion from these data are that the known ATP binding sites in $InsP_3R$-1, similar to $InsP_3R$-3, are not responsible for mediating the functional effects of ATP binding on Ca^{2+} release. Given the absence of any further known predicted nucleotide binding sites in the primary sequence, a novel ATP binding sequence must mediated these effects. A possibility is that the interaction is dependent on the tertiary structure of the $InsP_3R$. Alternatively, a cryptic ATP binding site may be exposed following the large conformational changes the receptor undergoes when binding Ca^{2+} [68-70]. Conceivably, the functional effects of ATP could also be mediated through a tightly bound accessory protein.

Mutagenesis of The $InsP_3R$ channel domain

The original prediction of six transmembrane (TM) domains in the C-terminal segment of the receptor based on hydropathy analysis has been confirmed experimentally [71-73]. Mutagenesis within the C-terminal channel domain (extending from ~amino-acid 2270-2749

of $InsP_3R$-1) has primarily been carried out to investigate the permeation/gating mechanisms of the channel [74-79], to study the role of the transmembrane domains in oligomerization [72, 80, 81], and to explore potential regulatory protein-protein interactions of the cytosol-exposed C-terminal tail [16, 82-84]. In general, studies on the conduction mechanism of $InsP_3R$ channels have been hampered by the lack of a detailed knowledge of the three dimensional structure of the channel domain, as well as the absence of convenient screening methods to analyze the behavior of large numbers of mutant channels. Hence, studies using mutagenesis approaches on the channel domain are quite limited. Figure 2A indicates the location of point mutations that have been engineered in the $InsP_3R$-1 pore domain together with their functional consequences. For comparison, the corresponding mutations in ryanodine receptors (RyR) are also shown. The information obtained from these mutations are summarized and discussed below.

Permeation and selectivity of $InsP_3R$ channels

A basic feature of $InsP_3R$ and related ryanodine receptor (RyR) channels are high divalent cation conductances (~50pS with 50 mM Ca^{2+}), even higher monovalent cation conductances (200-350pS with 140mM K^+), and only modest discrimination between divalent and monovalent ions (P_{Ca^{2+}/K^+} ~6-8) (reviewed in [25]). It has recently been suggested that the high K^+ conductance of RyR and $InsP_3R$ may yield an unexpected benefit in permitting these channels to provide their own countercurrent to compensate for the charge movement due to Ca^{2+} release from intracellular stores [85]. In contrast to K^+ channels [86] and voltage-gated Ca^{2+} channels [87], there is no compelling evidence for multi-ion occupancy of the channel, suggesting that electrostatic repulsion between ions residing in the pore is probably not the mechanism that accounts for the prodigious ion flux in $InsP_3R$ or RyR channels (but cf.[88]). This, and other biophysical properties, have led to the prediction that these channels would have short/wide pores with rings of negatively charged residues at either end to facilitate the rapid delivery and exit of ions through the pore [25, 89].

Our knowledge of $InsP_3R$ pore structure is primarily based on comparisons to the several K^+ channels for which crystal structures are available. The retention of channel activity in an $InsP_3R$ mutant from which TM 1-4 have been deleted strongly suggests that the pore of $InsP_3R$ is distal to TM 5 [75]. Within the primary sequence of this region a number of structural features common to many other channels can be recognized. These include sequentially: an outer helix (TM 5), a pore helix, a selectivity filter and an inner helix (TM 6). A homology model of the closed $InsP_3R$ pore has been constructed by da Fonseca and Morris using the KirBac1.1 K^+ channel as template [76] and is shown in Figure 2B. Several of the residues that are completely conserved in all three $InsP_3R$ and RyR isoforms within the pore-helix/ selectivity filter/ TM 6 segment have been mutated to alanine [76]. Not unexpectedly many of these mutations entirely eliminate channel function – presumably, as a result of disrupting critical structures such as the pore-helix or the selectivity filter. In particular, the removal of a negative charge at the luminal mouth of the receptor (D2550A) inactivates the channel. This mutant has been widely used by investigators as a “pore-dead” version of $InsP_3R$ [33, 90-94]. It is of interest that mutation of the analogous aspartate in RyR1 (D4899A) or RyR2 (D4831A) does not completely inactivate the channel [95-97], possibly because there is an adjacent glutamate residue that may fulfill a similar role. In addition, RyR possess a second pair of negative charges one residue away from the first pair, which in $InsP_3R$ are both positively charged residues (Figure 2A). The distribution of negative charges at the luminal entrance is therefore very different between the two channels and the implications of this for ion permeation and selectivity remain to be investigated. Surprisingly, the mutation of positively and negatively charged residues to alanine at the cytosolic exit of the channel has little impact on channel function with the exception of

R2596 which is the only charged residue that is conserved in all $InsP_3R$ and RyR isoforms (asterisk in Figure 2A).

The selectivity filter of the $InsP_3R$ channel has the "signature sequence" GGGVG with the valine replaced by an isoleucine in RyR channels. Mutation of the first three glycines to alanine inactivates the $InsP_3R$ channel whereas mutation of the fourth glycine does not [76]. Substitution of the bulky valine for the smaller isoleucine in $InsP_3R$ increased K^+ conductance to that seen in wild type RyR without changing selectivity [30, 33, 74]. It has been argued, based on modeling and molecular dynamics simulations, that the rather wide pore diameter of RyR (~7Å; [89]) and $InsP_3R$ (6.5Å; [98]) means that the selectivity filter actually exerts little effect on channel selectivity [99]. Instead, the density of negative charges at the luminal entrance has been proposed to play the major role in discriminating monovalent from divalent cations [89, 99, 100]. Some support for this hypothesis is the finding that the conservative substitution D2550E eliminated selectivity without altering K^+ conductance [74].

Some of the caveats of mutagenesis studies in $InsP_3R$ can be illustrated with specific examples. The positioning of a highly conserved positively charged residue (R2543) at the C-terminal end of the pore helix would not seem conducive to the rapid movement of positively charged ions through the channel. However, mutation of the residue to alanine has little effect in $^{45}Ca^{2+}$ flux assays, suggesting that the side-chain projects away from the permeation path as predicted by the homology model [74, 76]. Surprisingly, the R2543A mutation alters Ca^{2+} regulation of the receptor [74, 76]. That mutations within the pore of the channel can alter Ca^{2+} regulation is not without precedent in RyR studies [97] and could indicate that some point mutations can induce global conformational changes in the protein that have secondary regulatory effects. Another concern relates to the methods used to assay the function of $InsP_3R$ channels. Two glycine mutants in the selectivity filter were shown to be functional (G2549A) or non-functional (G2546A) in $^{45}Ca^{2+}$ flux assays and when anti-IgM was used to stimulate Ca^{2+} release in DT40 cell lines stably expressing the mutants [97]. However, both mutants were equally active when assayed by measuring K^+ conductance in patch clamp studies of the receptors expressed in plasma membranes of DT40 cells [97]. Thus the effects of mutagenesis on the conduction properties of the channel may differ if Ca^{2+} or K^+ is monitored as the permeant ion.

Gating mechanism of the channel

The binding of $InsP_3$ at the N-terminal ligand binding domain (LBD) induces a conformational change in the receptor [101, 102] but how this change is transmitted to the opening of the channel in the C-terminal segment of the protein is unknown. Early studies based on cross-linking and co-immunoprecipitation assays suggested that a 40 kDa N-terminal tryptic fragment on one monomeric subunit can be directly cross-linked to a C-terminal 95 kDa tryptic fragment of an adjacent subunit [103]. This supports the idea of a direct interaction between LBD and channel domains. Based on experiments with fusion proteins and mutagenesis studies of full-length receptors, the site of interaction in the channel domain has been narrowed to the cytosol-exposed loop between TM 4 and TM 5. This conclusion is supported by experiments in which the deletion of two 10 amino-acid segments, which together constitute the majority of the TM4,5-loop, disrupted channel function and the interaction between N and C-terminal tryptic fragments [77]. Point mutations indicate that the C-N interactions may involve polar residues particularly in the proximal portion of the TM4,5 loop [77]. By analogy with other channels [104-106] it has been proposed that the TM4,5 loop is an amphipathic helix lying parallel to the membrane that constricts the cytosolic face of the TM 6 pore-lining helix and therefore maintains the channel in its closed state [77]. The conformational change resulting from binding of $InsP_3$

leads to the mechanical movement of the TM4,5 loop that allows the TM 6 domains to separate and permits ion flux. A mutant $InsP_3R$ from which the TM 1-4 helices have been removed generates a constitutively open channel [75]. Although the TM 4,5 loop is retained in this mutant, it is unlikely to be oriented correctly to perform its normal function of compressing the TM 6 helices and this probably accounts for the constitutive activity of the mutant.

The N-terminal segments of the receptor that interact with the TM 4,5 loop have not been identified but a possible candidate is the suppressor domain (aa1-223). Deletion of this domain enhances $InsP_3$ binding to the receptor and eliminates channel function [79]. Interestingly, the TM4,5 loop deletion mutants also display enhanced $InsP_3$ binding, supporting the view that the loop and the LBD can influence each other [77]. Binding measurements of synthetic $InsP_3$ ligands to $InsP_3R$ constructs retaining or lacking the suppressor domain have led Taylor & colleagues [107] to propose that the energy from $InsP_3$ binding is used to re-arrange an intramolecular interaction between the suppressor domain and the inner ligand-binding core. Furthermore, they conclude that coupling to the channel occurs exclusively through the suppressor domain. This is consistent with the finding that mutations in the suppressor domain that diminish the ability of the suppressor domain to inhibit $InsP_3$ binding also decrease the open probability of the channel [107]. Despite these studies there is no direct experimental evidence that the suppressor domain is the N-terminal interaction site of the TM 4,5 loop. Alternative candidates could include other portions of the LBD, such as the α-helical domain (aa 427-605), which has the ability to gate endogenous $InsP_3R$ when targeted to ER membranes [108]. Uchida *et al*., reported [79] that deletion of a segment from the regulatory domain (aa 651-1130) interferes with channel function but this mutation also showed a large decrease in $InsP_3$ binding affinity which could be indicative of gross structural defects in the protein. Many of the mutations that have been used to study gating have involved large deletions of the full-length protein. A point mutation in Drosophila $InsP_3R$ (corresponding to S217F in the rat $InsP_3R$-1 sequence) shows an enhanced affinity for $InsP_3$ in Ca^{2+} flux assays without significant changes in $InsP_3$ binding affinity [78]. This mutation has therefore been characterized as a gain-of-function “gating” mutation. Additional point mutations in the suppressor or regulatory domains that specifically interfere with gating, without affecting $InsP_3$ binding, remain to be identified.

In common with other voltage-gated channels a major barrier to ion-flux is expected to be located at the constriction formed at the cytosolic end of the pore-lining TM 6 helices (Figure 2B). Identifying the residue(s) at the channel gate is problematic since the exact boundaries of the pore-lining helix have not been established. Many channels have bulky, hydrophobic residues at the gate and mutation of these residues to alanine disrupts channel function [109, 110]. There is a conserved phenylalanine residue at the end of the TM 6 helix that could potentially play such a role. However, the mutation F2592A does not inactivate the channel, suggesting that the gate of $InsP_3R$ may be located elsewhere [76]. Crystal structures of open K^+ channels suggest that bending of the inner pore-lining helix at a conserved glycine is part of the gating mechanism [109, 111]. A conserved glycine that could potentially function as a “gating hinge” is present in TM 6 but its mutation (G2586A) also does not inactivate the channel [76]. This suggests that if $InsP_3R$ possess a gating hinge it is probably not located at G2586. Nevertheless, the deliberate introduction of a bend in the helix with a G2586P substitution does produce a constitutively open channel [76]. It is possible that the TM 6 helix is inherently flexible and movement at a hinge may not be involved in the gating process, as suggested for the hERG K^+ channel [112].

Properties of the C-terminal tail

The C-terminal tail consists of ~160 amino-acids projecting into the cytosol from the putative boundary of the pore-lining TM 6 helix. Interestingly, the C-terminal tail of RyR are significantly shorter (~100 amino-acids) suggesting that this portion of the protein may have distinct functions in $InsP_3R$ and RyR. Initial studies indicated that deletion of the terminal 13 amino-acids from the $InsP_3R$ had no effect but deletion of 139 amino-acids eliminated channel function [16, 79]. A study using progressive deletion mutants found that channel function was lost somewhere between removal of 43 and 60 amino-acids. This study also showed that the C-tail contained a coiled-coil domain in the region being deleted and that a fusion protein of the coiled-coil could form tetramers *in vitro* [77]. Whether this occurs in full-length $InsP_3R$ *in vivo* is not known but there is evidence that the C-tail does contribute to the stability of the $InsP_3R$ tetramer [80]. Two highly conserved cysteine residues in the tail (C2610, C2613) have been identified whose mutation to serine or alanine inactivates the channel [76, 79]. The proximity of these residues to the terminus of the TM 6 helix has led to the suggestion that these residues may function as “gate keepers” [79]. The exact role played by the cysteines or the coiled-coil domain in channel function has not been established but it is likely that they are critical to maintaining the architecture of the C-terminal tail and providing a normal exit pathway for the conducting ion. In other channels [96, 113], including RyR [114], there is evidence for salt-bridge interactions between charged residues in the TM 4,5 loop and the C-tail. Additional approaches are required to assess whether this occurs in $InsP_3R$ and to determine how such interactions may contribute to the mechanics of the gating process.

The C-tail is also the site of interaction of a number of regulatory proteins (Figure 2C) and the phosphorylation site of Akt kinase (S2618) [47, 48] and Polo kinase (T2656) [115]. The interacting proteins cover a range of sizes and include: cytochrome c (12kDa, [116]), BclXl (26kDa, [84]), PP1α (37kDa, [16], Huntington-associated protein 1A (70kDa,[83]), 80K-H protein kinase C substrate (80kDa, [117]), G-protein coupled receptor kinase-interacting protein (95kDa; [118]) and 4.1N (98kDa; [119]). The localization of the binding sites to specific regions of the C-tail has been carried out using C-tail fusion proteins or yeast 2-hybrid assays. It is generally assumed that these relatively large proteins have free access to the entire length of the C-tail in the intact receptor. However, reaction of cysteine substitution mutants introduced into the C-tail of full length receptors with 5kDa PEG-maleimide suggest that freely accessible residues are limited to the terminal ~35 amino-acids. Using this assay, Ca^{2+}-induced conformational changes in the receptor were observed to enhance accessibility of the C-tail[68]. Structural information on the C-tail, particularly its orientation with respect to the membrane and the rest of the receptor, is needed to determine its role in $InsP_3R$ function and regulation.

Properties of the intraluminal loop

The intraluminal loop between TM 5 and the start of the pore-helix is ~62 amino-acids long in $InsP_3R$ compared to only ~14 amino-acids in RyR. Although the sequences show considerable variability between the three $InsP_3R$ isoforms, all three receptors contain 2 highly conserved cysteines in the loop (C2596, C2504 in $InsP_3R$-1). Mutation of neither cysteine affects channel function [120]. The loop has been proposed to be the binding site for ERp44, an ER luminal protein of the thioredoxin family that inhibits $InsP_3R$ channel function [120]. The binding appears to require both cysteines to be in their reduced state, although other determinants for binding must be involved since association with ERp44 is specific to the $InsP_3R$-1 isoform [120]. Recent studies have shown that the cysteines can form an intramolecular disulphide bond, at least in fusion proteins encoding the loop [121].

The disulphide bond status of the cysteines in full-length receptors and their role in redox regulation of $InsP_3R$ in intact cells remain to be evaluated.

Concluding Remarks

Clearly, analysis of mutant $InsP_3R$ family members using a combination of Ca^{2+} flux biochemical and electrophysiological techniques has yielded valuable information regarding $InsP_3R$ structure and function. Nevertheless, future molecular modeling based on more detailed knowledge of the 3 dimensional structure coupled with functional analysis of mutant receptors will undoubtedly be pivotal in addressing important remaining questions. Again, a fruitful approach may be to use related ion channels and shared protein binding partners as a framework for design of constructs. Obvious subjects to address include the sites of regulation of $InsP_3R$ by Ca^{2+}. Mutation of E2100 in $InsP_3R$ and corresponding conserved residues in RyR, significantly alters Ca^{2+} sensitivity of Ca^{2+} release [122, 123]. However, this single residue is unlikely to represent the sole determinant of the complex effects of Ca^{2+} on $InsP_3R$. Because of the critical importance of Ca^{2+} as a co-agonist at the $InsP_3R$, further defining regions of the protein responsible for this action is vital for an understanding of receptor function. By analogy with other Ca^{2+} binding motifs this will probably entail defining a pocket formed by coordinating residues within the 3 dimensional structure of the protein. Similarly, further work is also needed to gain a thorough understanding of the gating mechanism of the receptor, in particular an expanded characterization of the residues necessary for coupling $InsP_3$ binding to channel opening.

An additional approach is to exploit $InsP_3R$ isoform specific differences in structure and function by the generation of chimerical receptors. For example, this paradigm has been used to investigate the particular molecular motifs responsible for the distinct $InsP_3$ binding affinities and Ca^{2+} sensitivities exhibited by individual $InsP_3R$ isoforms [58, 124, 125]. An obvious further use for this approach is in elucidating legitimate nucleotide binding sites in $InsP_3R$-1 and $InsP_3R$-3. To this end an approach using the template of $InsP_3R$-2 ΔATPB, which is not regulated by ATP, [59] and serial substitution of portions of $InsP_3R$-1/-3 may be valuable in identifying the sites of nucleotide regulation of these isoforms.

References

1. Furuichi T, Yoshikawa S, Miyawaki A, Wada K, Maeda N, Mikoshiba K. Primary structure and functional expression of the inositol 1,4,5-trisphosphate-binding protein P400. Nature. 1989; 342:32–8. [PubMed: 2554142]
2. Mignery GA, Sudhof TC. The ligand binding site and transduction mechanism in the inositol-1,4,5-triphosphate receptor. Embo J. 1990; 9:3893–8. [PubMed: 2174351]
3. Yoshikawa F, Iwasaki H, Michikawa T, Furuichi T, Mikoshiba K. Cooperative formation of the ligand-binding site of the inositol 1,4, 5-trisphosphate receptor by two separable domains. The Journal of biological chemistry. 1999; 274:328–34. [PubMed: 9867847]
4. Yoshikawa F, Morita M, Monkawa T, Michikawa T, Furuichi T, Mikoshiba K. Mutational analysis of the ligand binding site of the inositol 1,4,5-trisphosphate receptor. The Journal of biological chemistry. 1996; 271:18277–84. [PubMed: 8663526]
5. Bosanac I, Alattia JR, Mal TK, Chan J, Talarico S, Tong FK, Tong KI, Yoshikawa F, Furuichi T, Iwai M, Michikawa T, Mikoshiba K, Ikura M. Structure of the inositol 1,4,5-trisphosphate receptor binding core in complex with its ligand. Nature. 2002; 420:696–700. [PubMed: 12442173]
6. Patel S, Joseph SK, Thomas AP. Molecular properties of inositol 1,4,5-trisphosphate receptors. Cell Calcium. 1999; 25:247–64. [PubMed: 10378086]
7. Patterson RL, Boehning D, Snyder SH. Inositol 1,4,5-trisphosphate receptors as signal integrators. Annu Rev Biochem. 2004; 73:437–65. [PubMed: 15189149]
8. Bezprozvanny I. The inositol 1,4,5-trisphosphate receptors. Cell Calcium. 2005; 38:261–72. [PubMed: 16102823]

9. Yamamoto H, Maeda N, Niinobe M, Miyamoto E, Mikoshiba K. Phosphorylation of P400 protein by cyclic AMP-dependent protein kinase and Ca2+/calmodulin-dependent protein kinase II. J Neurochem. 1989; 53:917–23. [PubMed: 2547906]
10. Walaas SI, Nairn AC, Greengard P. Regional distribution of calcium- and cyclic adenosine 3':5'-monophosphate-regulated protein phosphorylation systems in mammalian brain. I. Particulate systems. J Neurosci. 1983; 3:291–301. [PubMed: 6296331]
11. Walaas SI, Nairn AC, Greengard P. PCPP-260, a Purkinje cell-specific cyclic AMP-regulated membrane phosphoprotein of Mr 260,000. J Neurosci. 1986; 6:954–61. [PubMed: 3009734]
12. Danoff SK, Ferris CD, Donath C, Fischer GA, Munemitsu S, Ullrich A, Snyder SH, Ross CA. Inositol 1,4,5-trisphosphate receptors: distinct neuronal and nonneuronal forms derived by alternative splicing differ in phosphorylation. Proc Natl Acad Sci U S A. 1991; 88:2951–5. [PubMed: 1849282]
13. Ferris CD, Cameron AM, Bredt DS, Huganir RL, Snyder SH. Inositol 1,4,5-trisphosphate receptor is phosphorylated by cyclic AMP-dependent protein kinase at serines 1755 and 1589. Biochem Biophys Res Commun. 1991; 175:192–8. [PubMed: 1847804]
14. Nakade S, Rhee SK, Hamanaka H, Mikoshiba K. Cyclic AMP-dependent phosphorylation of an immunoaffinity-purified homotetrameric inositol 1,4,5-trisphosphate receptor (type I) increases Ca2+ flux in reconstituted lipid vesicles. The Journal of biological chemistry. 1994; 269:6735–42. [PubMed: 8120033]
15. Supattapone S, Danoff SK, Theibert A, Joseph SK, Steiner J, Snyder SH. Cyclic AMP-dependent phosphorylation of a brain inositol trisphosphate receptor decreases its release of calcium. Proc Natl Acad Sci U S A. 1988; 85:8747–50. [PubMed: 2847175]
16. Tang TS, Tu H, Wang Z, Bezprozvanny I. Modulation of type 1 inositol (1,4,5)-trisphosphate receptor function by protein kinase a and protein phosphatase 1alpha. J Neurosci. 2003; 23:403–15. [PubMed: 12533600]
17. Tertyshnikova S, Fein A. Inhibition of inositol 1,4,5-trisphosphate-induced Ca2+ release by cAMP-dependent protein kinase in a living cell. Proc Natl Acad Sci U S A. 1998; 95:1613–7. [PubMed: 9465064]
18. Wojcikiewicz RJ, Luo SG. Phosphorylation of inositol 1,4,5-trisphosphate receptors by cAMP-dependent protein kinase. Type I, II, and III receptors are differentially susceptible to phosphorylation and are phosphorylated in intact cells. The Journal of biological chemistry. 1998; 273:5670–7. [PubMed: 9488697]
19. Kubista H, Hawkins T, Moss SE. Characterisation of calcium signalling in DT40 chicken B-cells. Biochim Biophys Acta. 1998; 1448:299–310. [PubMed: 9920420]
20. Miyakawa T, Maeda A, Yamazawa T, Hirose K, Kurosaki T, Iino M. Encoding of Ca2+ signals by differential expression of IP3 receptor subtypes. Embo J. 1999; 18:1303–8. [PubMed: 10064596]
21. Sugawara H, Kurosaki M, Takata M, Kurosaki T. Genetic evidence for involvement of type 1, type 2 and type 3 inositol 1,4,5-trisphosphate receptors in signal transduction through the B-cell antigen receptor. Embo J. 1997; 16:3078–88. [PubMed: 9214625]
22. Wagner LE 2nd, Li WH, Yule DI. Phosphorylation of type-1 inositol 1,4,5-trisphosphate receptors by cyclic nucleotide-dependent protein kinases: a mutational analysis of the functionally important sites in the S2+ and S2- splice variants. The Journal of biological chemistry. 2003; 278:45811–7. [PubMed: 12939273]
23. Wagner LE 2nd, Li WH, Joseph SK, Yule DI. Functional consequences of phosphomimetic mutations at key cAMP-dependent protein kinase phosphorylation sites in the type 1 inositol 1,4,5-trisphosphate receptor. The Journal of biological chemistry. 2004; 279:46242–52. [PubMed: 15308649]
24. Tu H, Tang TS, Wang Z, Bezprozvanny I. Association of type 1 inositol 1,4,5-trisphosphate receptor with AKAP9 (Yotiao) and protein kinase A. The Journal of biological chemistry. 2004; 279:19375–82. [PubMed: 14982933]
25. Foskett JK, White C, Cheung KH, Mak DO. Inositol trisphosphate receptor Ca2+ release channels. Physiol Rev. 2007; 87:593–658. [PubMed: 17429043]

26. Mak DO, Foskett JK. Single-channel inositol 1,4,5-trisphosphate receptor currents revealed by patch clamp of isolated Xenopus oocyte nuclei. The Journal of biological chemistry. 1994; 269:29375–8. [PubMed: 7961913]
27. Mak DO, McBride S, Foskett JK. ATP regulation of type 1 inositol 1,4,5-trisphosphate receptor channel gating by allosteric tuning of Ca(2+) activation. The Journal of biological chemistry. 1999; 274:22231–7. [PubMed: 10428789]
28. Mak DO, McBride S, Foskett JK. Regulation by Ca2+ and inositol 1,4,5-trisphosphate (InsP3) of single recombinant type 3 InsP3 receptor channels. Ca2+ activation uniquely distinguishes types 1 and 3 insp3 receptors. J Gen Physiol. 2001; 117:435–46. [PubMed: 11331354]
29. Mak DO, McBride S, Raghuram V, Yue Y, Joseph SK, Foskett JK. Single-channel properties in endoplasmic reticulum membrane of recombinant type 3 inositol trisphosphate receptor. J Gen Physiol. 2000; 115:241–56. [PubMed: 10694253]
30. Dellis O, Dedos SG, Tovey SC, Taufiq UR, Dubel SJ, Taylor CW. Ca2+ entry through plasma membrane IP3 receptors. Science. 2006; 313:229–233. [PubMed: 16840702]
31. Dellis O, Rossi AM, Dedos SG, Taylor CW. Counting functional inositol 1,4,5-trisphosphate receptors into the plasma membrane. The Journal of biological chemistry. 2008; 283:751–5. [PubMed: 17999955]
32. Taylor CW, Dellis O. Plasma membrane IP3 receptors. Biochem Soc Trans. 2006; 34:910–2. [PubMed: 17052225]
33. Wagner LE 2nd, Joseph SK, Yule DI. Regulation of single inositol 1,4,5-trisphosphate receptor channel activity by protein kinase A phosphorylation. J Physiol. 2008; 586:3577–96. [PubMed: 18535093]
34. Komalavilas P, Lincoln TM. Phosphorylation of the inositol 1,4,5-trisphosphate receptor by cyclic GMP-dependent protein kinase. The Journal of biological chemistry. 1994; 269:8701–7. [PubMed: 8132598]
35. Komalavilas P, Lincoln TM. Phosphorylation of the inositol 1,4,5-trisphosphate receptor. Cyclic GMP-dependent protein kinase mediates cAMP and cGMP dependent phosphorylation in the intact rat aorta. The Journal of biological chemistry. 1996; 271:21933–8. [PubMed: 8702997]
36. Soulsby MD, Alzayady K, Xu Q, Wojcikiewicz RJ. The contribution of serine residues 1588 and 1755 to phosphorylation of the type I inositol 1,4,5-trisphosphate receptor by PKA and PKG. FEBS Lett. 2004; 557:181–4. [PubMed: 14741364]
37. Tertyshnikova S, Yan X, Fein A. cGMP inhibits IP3-induced Ca2+ release in intact rat megakaryocytes via cGMP- and cAMP-dependent protein kinases. J Physiol. 1998; 512(Pt 1):89–96. [PubMed: 9729619]
38. Schlossmann J, Ammendola A, Ashman K, Zong X, Huber A, Neubauer G, Wang GX, Allescher HD, Korth M, Wilm M, Hofmann F, Ruth P. Regulation of intracellular calcium by a signalling complex of IRAG, IP3 receptor and cGMP kinase Ibeta. Nature. 2000; 404:197–201. [PubMed: 10724174]
39. Bruce JI, Shuttleworth TJ, Giovannucci DR, Yule DI. Phosphorylation of inositol 1,4,5-trisphosphate receptors in parotid acinar cells. A mechanism for the synergistic effects of cAMP on Ca2+ signaling. The Journal of biological chemistry. 2002; 277:1340–8. [PubMed: 11694504]
40. Arguin G, Regimbald-Dumas Y, Fregeau MO, Caron AZ, Guillemette G. Protein kinase C phosphorylates the inositol 1,4,5-trisphosphate receptor type 2 and decreases the mobilization of Ca2+in pancreatoma AR4-2J cells. J Endocrinol. 2007; 192:659–68. [PubMed: 17332533]
41. Giovannucci DR, Groblewski GE, Sneyd J, Yule DI. Targeted phosphorylation of inositol 1,4,5-trisphosphate receptors selectively inhibits localized Ca2+ release and shapes oscillatory Ca2+ signals. The Journal of biological chemistry. 2000; 275:33704–11. [PubMed: 10887192]
42. Straub SV, Giovannucci DR, Bruce JI, Yule DI. A role for phosphorylation of inositol 1,4,5-trisphosphate receptors in defining calcium signals induced by Peptide agonists in pancreatic acinar cells. The Journal of biological chemistry. 2002; 277:31949–56. [PubMed: 12065595]
43. Soulsby MD, Wojcikiewicz RJ. The type III inositol 1,4,5-trisphosphate receptor is phosphorylated by cAMP-dependent protein kinase at three sites. The Biochemical journal. 2005; 392:493–7. [PubMed: 16107208]

44. Regimbald-Dumas Y, Arguin G, Fregeau MO, Guillemette G. cAMP-dependent protein kinase enhances inositol 1,4,5-trisphosphate-induced Ca2+ release in AR4-2J cells. J Cell Biochem. 2007; 101:609–18. [PubMed: 17203464]
45. Betzenhauser MJ, Fike JL, Wagner LE 2nd, Yule DI. Protein kinase A increases type-2 inositol 1,4,5-trisphosphate receptor activity by phosphorylation of serine 937. The Journal of biological chemistry. 2009; 284:25116–25. [PubMed: 19608738]
46. Maes K, Missiaen L, Parys JB, De Smet P, Sienaert I, Waelkens E, Callewaert G, De Smedt H. Mapping of the ATP-binding sites on inositol 1,4,5-trisphosphate receptor type 1 and type 3 homotetramers by controlled proteolysis and photoaffinity labeling. The Journal of biological chemistry. 2001; 276:3492–7. [PubMed: 11035010]
47. Khan MT, Wagner L 2nd, Yule DI, Bhanumathy C, Joseph SK. Akt kinase phosphorylation of inositol 1,4,5-trisphosphate receptors. The Journal of biological chemistry. 2006; 281:3731–7. [PubMed: 16332683]
48. Szado T, Vanderheyden V, Parys JB, De Smedt H, Rietdorf K, Kotelevets L, Chastre E, Khan F, Landegren U, Soderberg O, Bootman MD, Roderick HL. Phosphorylation of inositol 1,4,5-trisphosphate receptors by protein kinase B/Akt inhibits Ca2+ release and apoptosis. Proc Natl Acad Sci U S A. 2008; 105:2427–32. [PubMed: 18250332]
49. Villen J, Beausoleil SA, Gerber SA, Gygi SP. Large-scale phosphorylation analysis of mouse liver. Proc Natl Acad Sci U S A. 2007; 104:1488–93. [PubMed: 17242355]
50. Soulsby MD, Wojcikiewicz RJ. Calcium mobilization via type III inositol 1,4,5-trisphosphate receptors is not altered by PKA-mediated phosphorylation of serines 916, 934, and 1832. Cell Calcium. 2007; 42:261–70. [PubMed: 17257671]
51. Smith JB, Smith L, Higgins BL. Temperature and nucleotide dependence of calcium release by myo-inositol 1,4,5-trisphosphate in cultured vascular smooth muscle cells. The Journal of biological chemistry. 1985; 260:14413–6. [PubMed: 3877055]
52. Ehrlich BE, Watras J. Inositol 1,4,5-trisphosphate activates a channel from smooth muscle sarcoplasmic reticulum. Nature. 1988; 336:583–6. [PubMed: 2849060]
53. Maeda N, Kawasaki T, Nakade S, Yokota N, Taguchi T, Kasai M, Mikoshiba K. Structural and functional characterization of inositol 1,4,5-trisphosphate receptor channel from mouse cerebellum. J Biol Chem. 1991; 266:1109–1116. [PubMed: 1845986]
54. Ferris CD, Huganir RL, Snyder SH. Calcium flux mediated by purified inositol 1,4,5-trisphosphate receptor in reconstituted lipid vesicles is allosterically regulated by adenine nucleotides. Proc Natl Acad Sci U S A. 1990; 87:2147–51. [PubMed: 2156262]
55. Bezprozvanny I, Ehrlich BE. ATP modulates the function of inositol 1,4,5-trisphosphate-gated channels at two sites. Neuron. 1993; 10:1175–84. [PubMed: 7686381]
56. Iino M. Effects of adenine nucleotides on inositol 1,4,5-trisphosphate-induced calcium release in vascular smooth muscle cells. J Gen Physiol. 1991; 98:681–98. [PubMed: 1660060]
57. Maes K, Missiaen L, De Smet P, Vanlingen S, Callewaert G, Parys JB, De Smedt H. Differential modulation of inositol 1,4,5-trisphosphate receptor type 1 and type 3 by ATP. Cell Calcium. 2000; 27:257–67. [PubMed: 10859592]
58. Tu H, Wang Z, Nosyreva E, De Smedt H, Bezprozvanny I. Functional characterization of mammalian inositol 1,4,5-trisphosphate receptor isoforms. Biophysical journal. 2005; 88:1046–55. [PubMed: 15533917]
59. Betzenhauser MJ, Wagner LE 2nd, Iwai M, Michikawa T, Mikoshiba K, Yule DI. ATP modulation of Ca2+ release by type-2 and type-3 inositol (1, 4, 5)-triphosphate receptors. Differing ATP sensitivities and molecular determinants of action. The Journal of biological chemistry. 2008; 283:21579–87. [PubMed: 18505727]
60. Betzenhauser MJ, Wagner LE 2nd, Park HS, Yule DI. ATP regulation of type-1 inositol 1,4,5-trisphosphate receptor activity does not require walker Atype ATP-binding motifs. The Journal of biological chemistry. 2009; 284:16156–63. [PubMed: 19386591]
61. Park HS, Betzenhauser MJ, Won JH, Chen J, Yule DI. The type 2 inositol (1,4,5)-trisphosphate (InsP3) receptor determines the sensitivity of InsP3-induced Ca2+ release to ATP in pancreatic acinar cells. The Journal of biological chemistry. 2008; 283:26081–8. [PubMed: 18658132]

62. Maeda N, Niinobe M, Mikoshiba K. A cerebellar Purkinje cell marker P400 protein is an inositol 1,4,5-trisphosphate (InsP3) receptor protein. Purification and characterization of InsP3 receptor complex. Embo J. 1990; 9:61–7. [PubMed: 2153079]
63. Wierenga RK, Hol WG. Predicted nucleotide-binding properties of p21 protein and its cancer-associated variant. Nature. 1983; 302:842–4. [PubMed: 6843652]
64. Maes K, Missiaen L, Parys JB, Sienaert I, Bultynck G, Zizi M, De Smet P, Casteels R, De Smedt H. Adenine-nucleotide binding sites on the inositol 1,4,5-trisphosphate receptor bind caffeine, but not adenophostin A or cyclic ADP-ribose. Cell Calcium. 1999; 25:143–52. [PubMed: 10326681]
65. Wagner LE 2nd, Betzenhauser MJ, Yule DI. ATP binding to a unique site in the type-1 S2- inositol 1,4,5-trisphosphate receptor defines susceptibility to phosphorylation by protein kinase A. The Journal of biological chemistry. 2006; 281:17410–9. [PubMed: 16621795]
66. Tu H, Miyakawa T, Wang Z, Glouchankova L, Iino M, Bezprozvanny I. Functional characterization of the type 1 inositol 1,4,5-trisphosphate receptor coupling domain SII(+/-) splice variants and the Opisthotonos mutant form. Biophysical journal. 2002; 82:1995–2004. [PubMed: 11916857]
67. Lin C, Widjaja J, Joseph SK. The interaction of calmodulin with alternatively spliced isoforms of the type-I inositol trisphosphate receptor. The Journal of biological chemistry. 2000; 275:2305–11. [PubMed: 10644679]
68. Anyatonwu G, Khan MT, Schug ZT, da Fonseca PC, Morris EP, Joseph SK. Calcium dependent conformational changes in Inositol trisphosphate receptors. J Biol Chem. 2009
69. Hamada K, Miyata T, Mayanagi K, Hirota J, Mikoshiba K. Two-state conformational changes in inositol 1,4,5-trisphosphate receptor regulated by calcium. The Journal of biological chemistry. 2002; 277:21115–8. [PubMed: 11980893]
70. Hamada K, Terauchi A, Mikoshiba K. Three-dimensional rearrangements within inositol 1,4,5-trisphosphate receptor by calcium. The Journal of biological chemistry. 2003; 278:52881–9. [PubMed: 14593123]
71. Galvan DL, Borrego-Diaz E, Perez P, Mignery GA. Subunit oligomerization and topology of the inositol 1,4,5-trisphosphate receptor. J Biol Chem. 1999; 274:29483–29492. [PubMed: 10506212]
72. Joseph SK, Boehning D, Pierson S, Nicchitta CV. Membrane insertion, glycosylation and oligomerization of Inositol Trisphosphate receptors in a cell-free translation system. J Biol Chem. 1997; 272:1579–1588. [PubMed: 8999831]
73. Michikawa T, Hamanaka H, Otsu H, Yamamoto A, Miyawaki A, Furuichi T, Tashiro Y, Mikoshiba K. Transmembrane topology and sites of N-glycosylation of inositol 1,4,5-trisphosphate receptor. The Journal of biological chemistry. 1994; 269:9184–9. [PubMed: 8132655]
74. Boehning D, Mak DO, Foskett JK, Joseph SK. Molecular determinants of ion permeation and selectivity in inositol 1,4,5-trisphosphate receptor Ca2+ channels. The Journal of biological chemistry. 2001; 276:13509–12. [PubMed: 11278266]
75. Ramos-Franco J, Galvan D, Mignery G, Fill M. Location of the permeation pathway in the recombinant type-I Inositol 1,4,5-trisphosphate pathway. J Gen Physiol. 1999; 114:243–250. [PubMed: 10436000]
76. Schug ZT, da Fonseca PC, Bhanumathy CD, Wagner L, Zhang X, Bailey B, Morris EP, Yule DI, Joseph SK. Molecular characterization of the inositol 1,4,5-trisphosphate pore-forming segment. J Biol Chem. 2007; 283:2939–2948. [PubMed: 18025085]
77. Schug ZT, Joseph SK. The Role of the S4-S5 Linker and C-terminal Tail in Inositol 1,4,5-Trisphosphate Receptor Function. J Biol Chem. 2006; 281:24431–24440. [PubMed: 16815846]
78. Srikanth S, Wang Z, Tu H, Nair S, Mathew MK, Hasan G, Bezprozvanny I. Functional properties of the Drosophila melanogaster inositol 1,4,5-trisphosphate receptor mutants. Biophys J. 2004; 86:3634–3646. [PubMed: 15189860]
79. Uchida K, Miyauchi H, Furuichi T, Michikawa T, Mikoshiba K. Critical regions for activation gating of the inositol 1,4,5-trisphosphate receptor. J Biol Chem. 2003; 278:16551–16560. [PubMed: 12621039]
80. Galvan DL, Mignery GA. Carboxyl-terminal sequences critical for inositol 1,4,5-trisphosphate receptor subunit assembly. J Biol Chem. 2002; 277:48248–48260. [PubMed: 12377774]

81. Parker AK, Gergely FV, Taylor CW. Targeting of inositol 1,4,5-trisphosphate receptors to the endoplasmic reticulum by multiple signals within their transmembrane domains. The Journal of biological chemistry. 2004; 279:23797–805. [PubMed: 15033979]
82. Boehning D, Patterson RL, Sedaghat L, Glebova NO, Kurosaki T, Snyder SH. Cytochrome c binds to inositol (1,4,5) trisphosphate receptors, amplifying calcium-dependent apoptosis. Nat Cell Biol. 2003; 5:1051–1061. [PubMed: 14608362]
83. Tang TS, Tu H, Chan EY, Maximov A, Wang Z, Wellington CL, Hayden MR, Bezprozvanny I. Huntingtin and huntingtin-associated protein 1 influence neuronal calcium signaling mediated by inositol-(1,4,5) triphosphate receptor type 1. Neuron. 2003; 39:227–239. [PubMed: 12873381]
84. White C, Li C, Yang J, Petrenko NB, Madesh M, Thompson CB, Foskett JK. The endoplasmic reticulum gateway to apoptosis by Bcl-X(L) modulation of the InsP3R. Nat Cell Biol. 2005; 7:1021–8. [PubMed: 16179951]
85. Gillespie D, Fill MD. Intracellular calcium release channels mediate their own countercurrent: The ryanodine receptor case study. Biophysical journal. 2008
86. Doyle DA, Cabral JM, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R. The structure of the potassium channel: molecular basis of K+ conduction and selectivity. Science. 1998; 280:69–77. [PubMed: 9525859]
87. Ellinor PT, Yang J, Sather WA, Zhang J, Tsien RW. Ca2+ channel selectivity at a single locus for hih affinity interactions. Neuron. 1995; 15:1121–1132. [PubMed: 7576655]
88. Gillespie D, Xu L, Wang Y, Meissner G. (De)constructing the ryanodine receptor: modeling ion permeation and selectivity of the calcium release channel. J Phys Chem B. 2005; 109:15598–15610. [PubMed: 16852978]
89. Williams AJ, West DJ, Sitsapesan R. Light at the end of the Ca(2+)-release channel tunnel: structures and mechanisms involved in ion translocation in ryanodine receptor channels. Q Rev Biophys. 2001; 34:61–104. [PubMed: 11388090]
90. Alzayady KJ, Wojcikiewicz RJ. The role of Ca2+ in triggering inositol 1,4,5-trisphosphate receptor ubiquitination. Biochem J. 2005; 392:601–606. [PubMed: 16134970]
91. Bhanumathy CD, Nakao SK, Joseph SK. Mechanism of proteasomal degradation of inositol trisphosphate receptors in CHO-K1 cells. J Biol Chem. 2006; 281:3722–3730. [PubMed: 16316991]
92. Dellis O, Dedos SG, Tovey SC, Taufiq Ur R, Dubel SJ, Taylor CW. Ca2+ entry through plasma membrane IP3 receptors. Science. 2006; 313:229–33. [PubMed: 16840702]
93. Khan MT, Bhanumathy CD, Schug ZT, Joseph SK. Role of Inositol 1,4,5-trisphosphate receptors in apoptosis in DT40 lymphocytes. J Biol Chem. 2007; 282:32983–32990. [PubMed: 17875645]
94. van Rossum DB, Patterson RL, Kiselyov K, Boehning D, Barrow RK, Gill DL, Snyder SH. Agonist-induced Ca2+ entry determined by inositol 1,4,5-trisphosphate recognition. Proc Natl Acad Sci U S A. 2004; 101:2323–7. [PubMed: 14983008]
95. Chen SR, Li P, Zhao M, Li X, Zhang L. Role of the proposed pore-forming segment of the Ca2+ release channel (ryanodine receptor) in ryanodine interaction. Biophys J. 2002; 82:2436–2447. [PubMed: 11964232]
96. Du GG, Guo X, Khanna VK, MacLennan DH. Functional characterization of mutants in the predicted pore region of the rabbit cardiac muscle Ca(2+) release channel (ryanodine receptor isoform 2). J Biol Chem. 2001; 276:31760–31771. [PubMed: 11427530]
97. Gao L, Balshaw D, Xu L, Tripathy A, Xin C, Meissner G. Evidence for a role of the lumenal M3-M4 loop in skeletal muscle Ca2+ release channel (Ryanodine receptor) activity and conductance. Biophys J. 2000; 79:828–840. [PubMed: 10920015]
98. Marius P, Lea EJ, Dawson AP. Estimation of the pore size of the type-I IP3 receptor reconstituted in single unilamellar vesicles. Biophysical journal. 1980
99. Welch W, Rheault S, West DJ, Williams AJ. A model of the putative pore region of the cardiac ryanodine receptor channel. Biophys J. 2004; 87:2335–2351. [PubMed: 15454434]
100. Xu L, Wang Y, Gillespie D, Meissner G. Two rings of negative charges in the cytosolic vestibule of type-I ryanodine receptors modulate ion fluxes. Biophysical journal. 2006; 90:443–453. [PubMed: 16239337]

101. Chan J, Whitten AE, Jeffries CM, Bosanac I, Mal TK, Ito J, Porumb H, Michikawa T, Mikoshiba K, Trewhella J, Ikura M. Ligand-induced conformational changes via flexible linkers in the amino-terminal region of the inositol 1,4,5-trisphosphate receptor. J Mol Biol. 2007; 373:1269–1280. [PubMed: 17915250]
102. Mignery G, Sudhof TC. Molecular analysis of inositol 1,4,5-trisphosphate receptors. Methods in Neurosciences. 1993; 18:247–265.
103. Boehning D, Joseph SK. Direct association of ligand-binding and pore domains in homo- and heterotetrameric inositol 1,4,5-trisphosphate receptors. EMBO J. 2000; 19:5450–5459. [PubMed: 11032812]
104. Decher N, Chen J, Sanguinetti MC. Voltage-dependent gating of hyperpolarization-activated, cyclic nucleotide-gated pacemaker channels: molecular coupling between the S4-S5 and C-linkers. J Biol Chem. 2004; 279:13859–13865. [PubMed: 14726518]
105. Ferrer T, Rupp J, Piper DR, Tristani-Firouzi M. The S4-S5 linker directly couples voltage sensor movement to the activation gate in the human ether-a'-go-go-related gene (hERG) K+ channel. J Biol Chem. 2006; 281:12858–12864. [PubMed: 16524878]
106. Long SB, Campbell EB, MacKinnon R. Voltage sensor of Kv1.2: structural basis of electromechanical coupling. Science. 2005; 309:903–908. [PubMed: 16002579]
107. Rossi AM, Riley AM, Tovey SC, Rahman T, Dellis O, Taylor EJ, Veresov VG, Potter BV, Taylor CW. Synthetic partial agonists reveal key steps in IP3 receptor activation. Nat Chem Biol. 2009; 5:631–639. [PubMed: 19668195]
108. Varnai P, Balla A, Hunyady L, Balla T. Targeted expression of the inositol 1,4,5-triphosphate receptor (IP3R) ligand-binding domain releases Ca2+ via endogenous IP3R channels. Proc Natl Acad Sci U S A. 2005; 102:7859–7864. [PubMed: 15911776]
109. Kuo A, Gulbis JM, Antcliff JF, Rahman T, Lowe ED, Zimmer J, Cuthbertson J, Ashcroft FM, Ezaki T, Doyle DA. Crystal structure of the potassium channel KirBac1.1 in the closed state. Science. 2003; 300:1922–1926. [PubMed: 12738871]
110. Rojas A, Wu J, Wang R, Jiang C. Gating of the ATP-sensitive K+ channel by a pore-lining phenylalanine residue. Biochim Biophys Acta. 2007; 1768:39–51. [PubMed: 16970907]
111. Jiang Y, Lee A, Chen J, Cadene M, Chait BT, MacKinnon R. The open pore conformation of potassium channels. Nature. 2002; 417:523–526. [PubMed: 12037560]
112. Hardman RM, Stansfeld PJ, Dalibalta S, Sutcliffe MJ, Mitcheson JS. Activation gating of hERG potassium channels: S6 glycines are not required as gating hinges. J Biol Chem. 2007; 282:31972–31981. [PubMed: 17823114]
113. Tristani-Firouzi M, Chen J, Sanguinetti MC. Interactions between S4-S5 linker and S6 transmembrane domain modulate gating of HERG K+ channels. J Biol Chem. 2002; 277:18994–19000. [PubMed: 11864984]
114. Lee EH, Allen PD. Homo-dimerization of RyR1 C-terminus via charged residues in random coils or in an alpha-helix. Exp Mol Med. 2007; 39:594–602. [PubMed: 18059135]
115. Vanderheyden V, Wakai T, Bultynck G, De Smedt H, Parys JB, Fissore RA. Regulation of inositol 1,4,5-trisphosphate receptor type 1 function during oocyte maturation by MPM-2 phosphorylation. Cell Calcium. 2009; 46:56–64. [PubMed: 19482353]
116. Boehning D, van Rossum DB, Patterson RL, Snyder SH. A peptide inhibitor of cytochrome c/inositol 1,4,5-trisphosphate receptor binding blocks intrinsic and extrinsic cell death pathways. Proc Natl Acad Sci U S A. 2005; 102:1466–1471. [PubMed: 15665074]
117. Kawaai K, Hisatsune C, Kuroda Y, Mizutani A, Tashiro T, Mikoshiba K. 80K-H interacts with inositol 1,4,5-trisphosphate (IP3) receptors and regulates IP3-induced calcium release activity. J Biol Chem. 2009; 284:372–380. [PubMed: 18990696]
118. Zhang S, Hisatsune C, Matsu-ura T, Mikoshiba K. G-protein-coupled receptor kinase-interacting proteins inhibit apoptosis by inositol 1,4,5-triphosphate receptor-mediated Ca2+ signal regulation. J Biol Chem. 2009; 284:29158–29169. [PubMed: 19706611]
119. Fukatsu K, Bannai H, Inoue T, Mikoshiba K. 4.1N binding regions of inositol 1,4,5-trisphosphate receptor type 1. Biochem Biophys Res Commun. 2006; 342:573–6. [PubMed: 16487933]

120. Higo T, Hattori M, Nakamura T, Natsume T, Michikawa T, Mikoshiba K. Subtype-specific and ER lumenal environment-dependent regulation of inositol 1,4,5-trisphosphate receptor type 1 by ERp44. Cell. 2005; 120:85–98. [PubMed: 15652484]

121. Kang S, Kang J, Kwon H, Frueh D, Yoo SH, Wagner G, Park S. Effects of redox potential and Ca2+ on Inositol 1,4,5-trisphosphate receptor L3-1 loop region: implications for receptor regulation. J Biol Chem. 2008; 283:25567–25575. [PubMed: 18635540]

122. Tu H, Nosyreva E, Miyakawa T, Wang Z, Mizushima A, Iino M, Bezprozvanny I. Functional and biochemical analysis of the type 1 inositol (1,4,5)-trisphosphate receptor calcium sensor. Biophysical journal. 2003; 85:290–9. [PubMed: 12829484]

123. Miyakawa T, Mizushima A, Hirose K, Yamazawa T, Bezprozvanny I, Kurosaki T, Iino M. Ca(2+)-sensor region of IP(3) receptor controls intracellular Ca(2+) signaling. Embo J. 2001; 20:1674–80. [PubMed: 11285231]

124. Iwai M, Michikawa T, Bosanac I, Ikura M, Mikoshiba K. Molecular basis of the isoform-specific ligand-binding affinity of inositol 1,4,5-trisphosphate receptors. The Journal of biological chemistry. 2007; 282:12755–64. [PubMed: 17327232]

125. Tu H, Wang Z, Bezprozvanny I. Modulation of mammalian inositol 1,4,5-trisphosphate receptor isoforms by calcium: a role of calcium sensor region. Biophysical journal. 2005; 88:1056–69. [PubMed: 15531634]

126. Joseph SK, Pierson S, Samanta S. Trypsin digestion of the inositol trisphosphate receptor: implications for the conformation and domain organization of the protein. The Biochemical journal. 1995; 307(Pt 3):859–65. [PubMed: 7741718]

127. Yoshikawa F, Iwasaki H, Michikawa T, Furuichi T, Mikoshiba K. Trypsinized cerebellar inositol 1,4,5-trisphosphate receptor. Structural and functional coupling of cleaved ligand binding and channel domains. The Journal of biological chemistry. 1999; 274:316–27. [PubMed: 9867846]

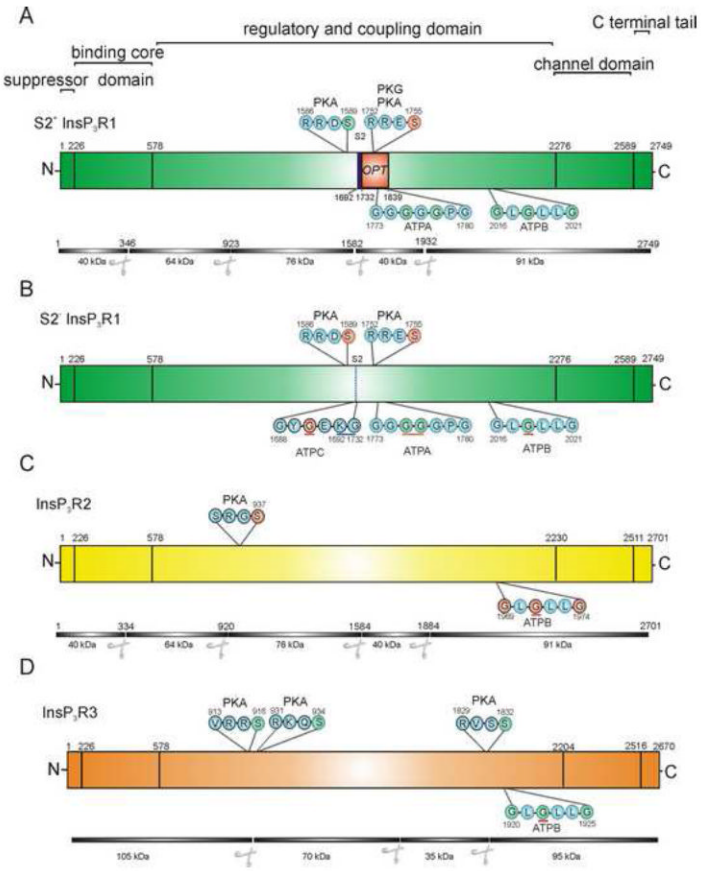

Figure 1. Consensus PKA phosphorylation substrate motifs and "Walker type A" ATP binding motifs in the InsP$_3$R isoforms

The general domain structure of InsP$_3$R family members is shown for S2$^+$ InsP$_3$R1 in A; S2$^-$ InsP$_3$R-1 in B; InsP$_3$R-2 in C and InsP$_3$R-3 in D. In the schematic for each receptor PKA phosphorylation sites are shown above and putative ATP binding domains are shown below. Residues colored red indicate amino acids which when mutated have functional consequences. Residues colored in green indicate amino acids which when mutated did not result in effects on InsP$_3$R activity. Residues within the putative ATP binding domains underlined in red indicate residues which abrogate binding of ATP to GST-fusion proteins containing the particular site. In InsP$_3$R-1 (A and B) the PKA phosphorylation sites Serine 1589 and Serine 1755 are separated by the S2 splice region shown in blue (amino acids 1692-1732) in the top panel. In B the blue underlined residues indicate the N and C terminal boundaries of the S2 splice region. The site of the in frame deletion present in the *Opthisthotonos* mouse (*OPT*; amino acids 1732-1839) which results in deletion of a phosphorylation site and the ATPA site is indicated. Below the schematic is a diagram of the fragments of InsP$_3$R-1 produced by limited digestion with trypsin [46, 126, 127]. ATP binding was reported in the C-terminal 40 kDa and 91 kDa fragments [46]. In B, PKA sites and the complement of ATP binding motifs are shown in the S2$^-$ InsP$_3$R-1. The amino acid numbering is based on the rat S2$^+$ InsP$_3$R-1. In C, a functionally important PKA phosphorylation site at Serine 937 is shown. The ATPB Walker motif is also functionally relevant in InsP$_3$R-2. Below the schematic a diagram is shown which illustrates a subcloning strategy used to determine phosphorylation sites in InsP$_3$R-2. The fragments of InsP$_3$R2 were based on tryptic sites in InsP$_3$R-1. PKA phosphorylation of the 76 kDa fragment was demonstrated [45]. In D, phosphorylation sites Serine 916, Serine 934 and Serine 1832 are

shown, together with the ATPB Walker motif. Mutation of each of these sites appears to be without functional consequence. Below the schematic is a diagram depicting fragments produced by limited chymotryptic digestion of $InsP_3R$-3 [43, 46]. ATP binding was demonstrated in the C-terminal 95 kDa fragment [46]. PKA dependent phosphorylation was shown in the N terminal 105 kDa fragment; the 70 kDa and 35 kDa fragments [43].

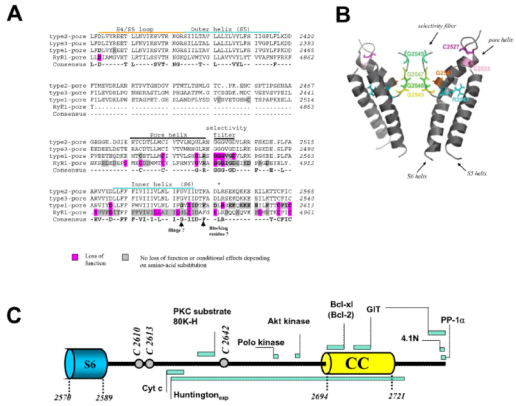

Figure 2. The pore and C-terminal tail of $InsP_3$ receptors

Panel A shows the sequence of a portion of the pore region of $InsP_3$Rs. The consensus residues shown in bold are identical in all 3 $InsP_3$R isoforms and all 3 RyR isoforms although only the sequence of RyR1 is shown in the Figure. Residues which have been mutated in $InsP_3$R1 are shown together with their functional consequences. The analogous mutations in RyRs are shown. For additional information see text and Ref. (9). The R2596 position is indicated by an asterisk. *Panel B* A homology model of the $InsP_3$R based on a KirBac 1.1 template is shown. The image is taken from Ref. (9) and is used with permission. Only the location of selected mutations from Panel A are shown. The linkers between S5 and the pore helix, and between the selectivity filter and S6, were not included in the model. *Panel C* shows a cartoon of the C-terminal tail with endogenous cysteines indicated by open circles and bars to indicate the approximate location of interacting proteins. CC = coiled coil domain. GIT; G-protein coupled receptor kinase-interacting protein, PP1-a; protein phosphates 1-a, 4.1N; neuron specific isoform of erythrocyte protein band 4.1. For additional details see text.