Available online at www.sciencedirect.com

SCIENCE DIRECT

Biochimica et Biophysica Acta 1742 (2004) 89–102

BIOCHIMICA ET BIOPHYSICA ACTA BBA

http://www.elsevier.com/locate/bba

Review

Structural insights into the regulatory mechanism of IP_3 receptor

Ivan Bosanac[a], Takayuki Michikawa[b,c], Katsuhiko Mikoshiba[b,c,d,e], Mitsuhiko Ikura[a,*]

[a]*Division of Molecular and Structural Biology, Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, 610 University Avenue, Toronto, Ontario, Canada M5G 2M9*

[b]*Division of Molecular Neurobiology, Department of Basic Medical Sciences, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan*

[c]*Calcium Oscillation Project, International Cooperative Research Project, Japan Science and Technology Corporation, Tokyo 108-0071, Japan*

[d]*Laboratory for Developmental Neurobiology, Brain Science Institute, RIKEN, Saitama 351-0198, Japan*

[e]*Division of Neural Signal Information NTT-IMSUT, Department of Basic Medical Sciences, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan*

Received 24 August 2004; received in revised form 10 September 2004; accepted 14 September 2004
Available online 27 October 2004

Abstract

Inositol 1,4,5-trisphosphate receptors (IP_3R) are intracellular Ca^{2+} release channels whose opening requires binding of two intracellular messengers IP_3 and Ca^{2+}. The regulation of IP_3R function has also been shown to involve a variety of cellular proteins. Recent biochemical and structural analyses have deepened our understanding of how the IP_3-operated Ca^{2+} channel functions. Specifically, the atomic resolution structure of the IP_3-binding region has provided a sound structural basis for the receptor interaction with the natural ligand. Electron microscopic studies have also shed light on the overall shape of the tetrameric receptor. This review aims to provide comprehensive overview of the current information available on the structure and function relationship of IP_3R.

Keywords: Calcium signaling; IP_3; IP_3 receptor structure

1. Introduction

Numerous physiological processes are linked to an increase in cytosolic calcium (Ca^{2+}) concentrations, through either Ca^{2+} intake from the extracellular environment, or the release from the internal Ca^{2+} stores. Ca^{2+} release from the endoplasmic reticulum (ER) is activated by the second messenger inositol 1,4,5-trisphosphate (IP_3), a product of phosphatidylinositol 4,5-bisphosphate hydrolysis through either the G protein-coupled receptor/phospholipase C (PLC)-β, or the tyrosine kinase receptor/PLC-γ signaling pathway [1]. IP_3 functions by binding to the membrane-associated IP_3 receptors (IP_3R) [1–3]. Binding of IP_3 to the receptor increases its sensitivity to Ca^{2+}, and only after Ca^{2+} is bound can trafficking of the Ca^{2+} into the cytosol take place [4,5]. Notably, Ca^{2+} has a biphasic action on the IP_3R with a stimulatory effect at low Ca^{2+} concentrations and an inhibitory effect at higher Ca^{2+} concentrations [4,5]. Acting as a signal transducer between two ubiquitous second messengers IP_3 and Ca^{2+}, IP_3R has been implicated in a variety of cellular and physiological processes as diverse as cell division, cell proliferation, apoptosis, fertilization, development, behavior, memory and learning.

In mammals, there are three distinct types of IP_3R with splice variants observed among the types [6,7]. Functional Ca^{2+} channels are formed from homo- or heterotetramer arrangements of these IP_3Rs [7]. The most widely studied IP_3R is type 1 (IP_3R1), which is found in high abundance in cerebellar Purkinje cells of the central nervous system [8,9]. Since functional domain organization is the same for all three types of IP_3R, for the purpose of this review, mouse IP_3R1 (mIP_3R1, GenBank accession no. X15373) is used as a reference.

* Corresponding author. Tel.: +1 416 946 4501; fax: +1 416 946 2055/6529.
E-mail address: mikura@uhnres.utoronto.ca (M. Ikura).

0167-4889/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2004.09.016

Numerous biochemical analyses of IP_3R and its interaction with other cellular proteins and small molecules have advanced our knowledge of IP_3R function as a Ca^{2+} release channel. Regulation of IP_3R is quite complex and a detailed understanding of mechanism(s) underlying the channel function requires further analysis from both a functional and structural viewpoint. Structural studies of the entire tetrameric receptor, or its segments, have made a significant contribution to this research. A number of electron microscopic (EM)-derived 3D reconstructions of IP_3R, recently obtained with a resolution around 30 Å [10–14], have provided information about gross arrangement of the receptor. Presently, a high-resolution structure of the IP_3R is available only for the IP_3-binding core in complex with IP_3 [15]. The aim of this review is to highlight structural information available on the receptor and to discuss its structure–function relationship. Emphasis is placed on the N-terminal ligand-binding region, where the richest structural information is available and many interaction molecules bind. The structures of the channel domain will be discussed on the basis of the crystal structure of potassium channels and the homology to ryanodine receptor (RyR), a close relative of IP_3Rs.

2. Molecular architecture

There are three functionally distinct regions within the mIP_3R1, a polypeptide of 2749 residues [16] (Fig. 1A): the N-terminal portion of the receptor capable of binding IP_3, the channel-forming region localized to the C-terminus and the regulatory segment connecting the two termini [17,18]. In order to understand the structure–function relationship of these three regions of the receptor, further attempts have been made to dissect more precise domains with specific molecular functions.

Employing deletion mutagenesis, the minimal region required for binding of IP_3 (i.e. IP_3-binding core) was mapped to residues 226–578 [19]. The domain encompassed by the first 223 residues was implicated in

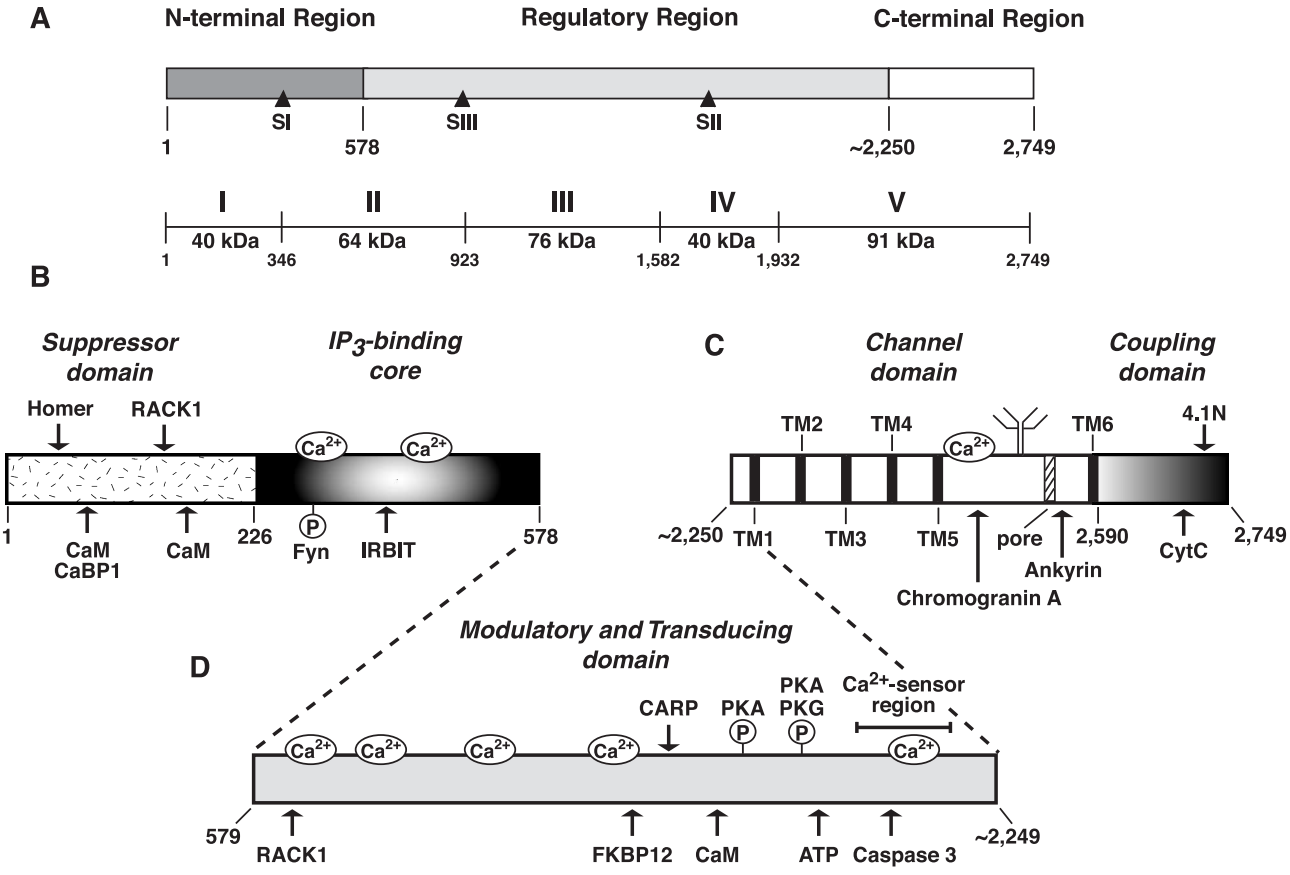

Fig. 1. Overall domain architecture of IP_3R1. (A) The three functional regions of IP_3R and the location of the three splice sites (SI–SIII). Boundaries of the five protein fragments generated from limited trypsin digestion are shown below. (B) Location of the suppressor domain and the IP_3-binding core within the N-terminal region of the receptor. Possible binding sites of the interacting partners for both domains are shown with arrows. (C) The C-terminal portion of the receptor consists of the channel domain and the coupling domain. Six transmembrane segments (vertical solid bars), the pore-forming region (diagonally striped box) and two glycosylation sites (tree-like structure) are shown together with the potential binding partner sites. (D) Modulatory and transducing domain with suggested docking sites for its binding partners. Association of myosin, CaMKII, BANK, IRAG, sigma-1 receptor and calcineurin have been reported, however, interacting regions of the IP_3R are not well known at this time (as reviewed in Ref. [78]).

inhibition of IP_3-binding to the core domain, and thus was named the suppressor domain [19] (Fig. 1B). The six transmembrane (TM) helices responsible for creating the channel reside within residues 2276 to 2589 [20]. The remaining 160 residues of the C-terminus comprise a coupling domain [21] (Fig. 1C). The modulatory and transducing domain [21], which occupies the central portion of the receptor, is involved in binding small molecules and other proteins as well as transferring signal from the ligand-binding domain to the channel domain (Fig. 1D). Five out of the eight Ca^{2+}-binding sites are localized to the modulatory and transducing domain, two are found within the IP_3-binding core, and one within the channel domain [22–24]. A binding site for an ATP molecule [25,26] and the presence of two phosphorylation sites [27–30] were also detected in the modulatory domain. In addition, CaM [31,32], FKBP12 [33–35], CARP [36], and caspase 3 [37] all have binding sites within this region of the receptor. The suppressor domain is proposed to bind CaM [32,38,39], CaBP1 [40,41], Homer [42] and RACK1 [43], whereas chromogranin A [44,45], ankyrin [46], CytC [47] and 4.1N [48,49] protein binding was detected in the coupling domain. A Fyn phosphorylation site [50] and an IRBIT binding site [51] were proposed within the IP_3-binding core (Fig. 1B).

3. IP_3-binding core

Early deletion mutagenesis assigned the IP_3-binding site to the N-terminus of the receptor within the first 650 residues [17,18]. Employing photoaffinity ligands, Mourey et al. [52] later showed that residues 476–501 were involved in this interaction. With more systematic deletion mutagenesis, the minimal region required for binding of IP_3 was mapped between residues 226 and 578 [19]. Taking into account the three phosphate groups of the IP_3 molecule, positively charged residues were suspected of being involved in the docking of IP_3 to the receptor. This proposal was supported by extensive site-directed mutagenesis which revealed that a number of highly conserved Arg and Lys residues were required for ligand docking, with R265, K508 and R511 being essential [19]. Binding analysis (as reviewed in Ref. [53]) with different forms of inositol phosphates determined that phosphate groups at position 4 and 5 (P4 and P5, respectively), and a hydroxyl group at position 6, were key to the interaction with the receptor. Using chemically crosslinked dimeric derivatives of IP_3, Riley et al. [54,55] estimated the distance between the two ligand binding sites within the channel to be as short as 15 Å.

3.1. Crystal structure of the IP_3-binding core

The atomic resolution structure of the IP_3-binding core (IP_3R1_{core}) in complex with IP_3 was solved recently by X-ray crystallography to a 2.2 Å resolution [15] (Fig. 2A). This portion of the receptor is comprised of two asymmetric domains (β-domain and α-domain) oriented perpendicularly to each other. At the interface of these structurally distinct domains, a highly positive-charged pocket is created to which the IP_3 molecule binds. Coordination of P5 (R265, R269, R504, K508, R511 and Y567) involves residues primarily from the α-domain. In contrast, the P4 coordination (R265, T266, T267, G268, R269 and K569) is predominately done by the β-domain residues (Fig. 2B). P1 only interacts with R568 and K569, supporting the less critical role of P1 over P4 and P5 [56,57]. Interestingly, non-basic residues (T266, T267, G268 and Y567) were also involved in ligand interaction [15]. All the basic residues involved in IP_3 coordination were consistent with previous mutagenesis [19].

Two highly conserved areas, P-I and P-II, were identified on the surface of the IP_3R1_{core}. The P-I surface overlaps with the IP_3 binding site and extends towards the C-terminus of the α-domain [15]. The P-II surface, which contains many acidic residues, is localized at the hinge between the two domains and is more hydrophilic in nature than the P-I region. The P-II region may serve as a possible Ca^{2+}-binding site (Ca-II, residues E283, E285, D444, and D448) with the ability to coordinate two Ca^{2+} ions (Fig. 2D). Another potential Ca^{2+}-binding site was identified within the β-domain (Ca-I, residues E246, E425, D426, and E428) [15] (Fig. 2C). The presence of structurally possible Ca^{2+}-binding sites within this portion of the receptor agrees with a previous deletion mutagenesis study [24], which detected Ca^{2+} binding to residues 304–381 and residues 378–450. Furthermore, site-directed mutagenesis of residues E425, D426, E428, D442, and D444 to alanine abolished Ca^{2+} binding [39]. Implications of these Ca^{2+}-binding sites in the channel activation mechanism will be discussed below.

The lack of a ligand-free IP_3R1_{core} structure precludes detailed discussion on IP_3-induced structural changes in the core domain. However, our preliminary NMR studies indicated that, while IP_3-loaded IP_3R1_{core} yielded a well resolved ^{1}H-^{15}N TROSY-HSQC spectrum containing a large number of peaks, the removal of IP_3 from IP_3R1_{core} dramatically impaired the quality of the spectrum, resulting in a large number of broadened peaks (Fig. 3C and D). These results strongly suggest a dynamic equilibrium exists between two or more conformations in the apo state of IP_3R1_{core}, presumably due to the hinge motion around the linker region (residues 434–437) between the β-domain and the α-domain (Fig. 3A and B).

Modeling of the interaction of adenophostin A, the most potent IP_3R agonist, with the IP_3R_{core} was carried out on the basis of the structure of the IP_3R_{core}–IP_3 complex [15]. The resulting structure showed the agonist's interaction with the receptor to be very similar to that of IP_3 interaction. The P4 and P5 of IP_3 are mimicked by the glucose 3″,4″-bisphosphate of adenophostin A, whereas P1

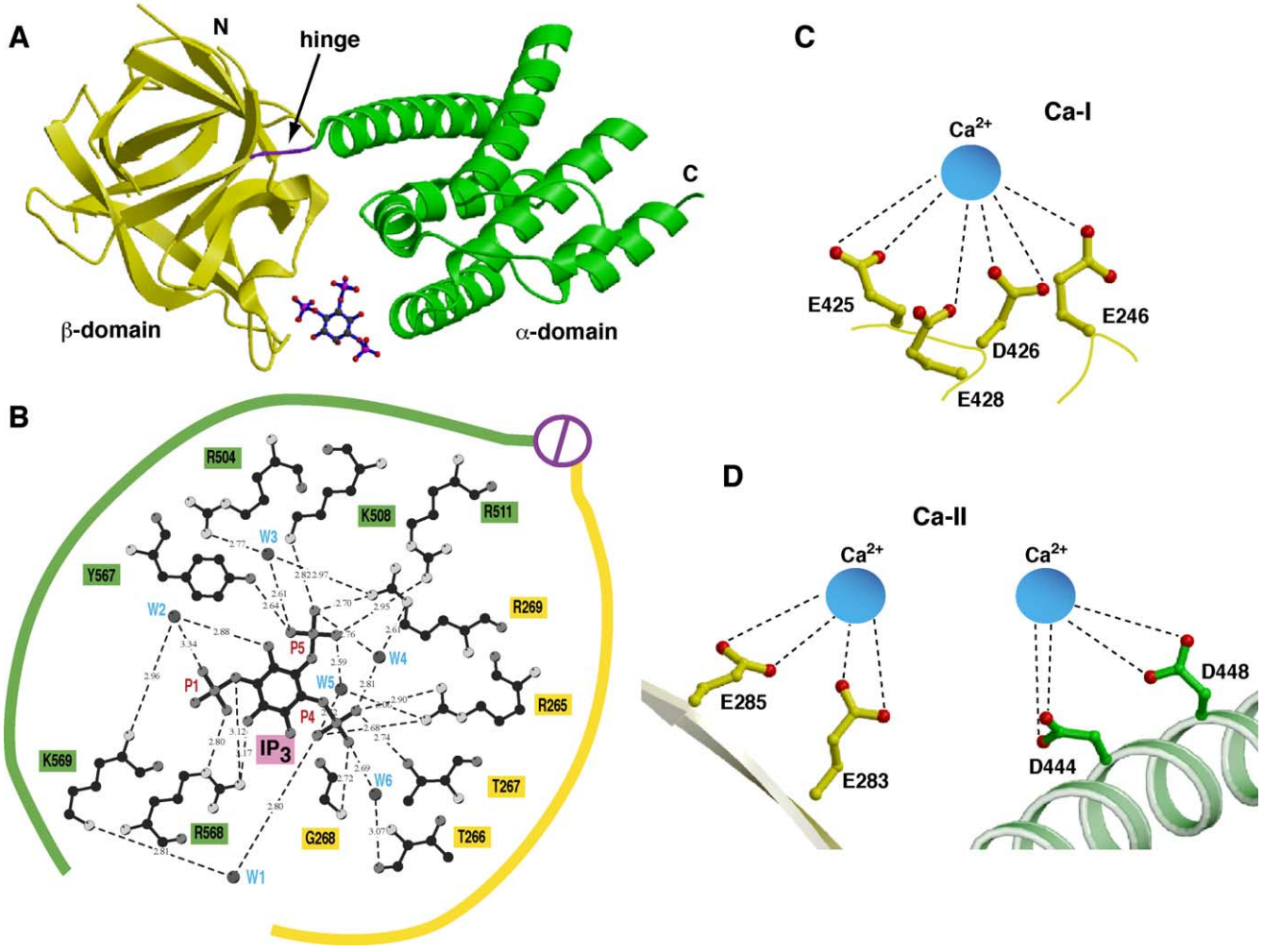

Fig. 2. Structure of IP_3R_{core} in complex with IP_3. (A) Ribbon diagram of the IP_3R_{core} complex with the β-domain (yellow, residues 224–434), the α-domain (green, residues 438–604) and the hinge region (purple, residues 435–437). The IP_3 molecule (carbons in gray, phosphate in purple and oxygens in red) lies in the interface between the two domains. (B) Coordination of IP_3 by IP_3R1_{core}. Residues from the α-domain and β-domain are highlighted in green and yellow, respectively. Water molecules are in cyan and phosphate groups are in red. IP_3 is highlighted in pink. The hinge region is shown in purple. (C and D) Hypothetical coordination of Ca^{2+} ion(s) in Ca-I (C) and Ca-II sites (D). The orientation of the Asp and Glu side-chains must change upon binding to Ca^{2+} ions.

is mimicked by the 2′-phosphate group of adenophostin A [58].

4. Suppressor domain

High-affinity IP_3 binding to the ligand binding core (226–578) is reduced to a physiological range by the N-terminal 223 residues [19], hence called the suppressor domain (IP_3R1_{sup}). Involvement of IP_3R_{sup} in IP_3-suppression is clearly illustrated in IP_3 binding studies performed on protein fragments encompassing different portions of the N-terminus of the receptor. The construct encompassing IP_3R1_{core} and IP_3R1_{sup} (i.e. residues 1–604) showed a reduced affinity for IP_3 (K_d~45 nM) [59] compared to the constructs containing only IP_3R1_{core} (residues 224–579, K_d~2.3 nM) [19]. Moreover, residues 1–223 were observed to be critical for IP_3-induced gating, since receptors lacking this region can bind IP_3 with a higher affinity than the native receptor, but cannot open the channel [21]. Presently, no structural information is available for IP_3R1_{sup}. Analysis of the N-terminal regions of IP_3R and RyR by Ponting [60] suggested that this portion of IP_3R and RyR sequences possesses some similarity to the core domain.

4.1. Possible mechanisms for IP_3 binding attenuation by suppressor domain

The crystal structure of the IP_3-binding core provided invaluable insights into IP_3 coordination by the receptor. The mechanism underlying the action of the IP_3R1_{sup} remains, however, to be determined. From the current information available, one can speculate that the inhibition of IP_3 binding may involve interaction between the IP_3R1_{sup} and the IP_3R1_{core} such that the IP_3-binding site on the core domain is not accessible to free IP_3 (Fig. 3E).

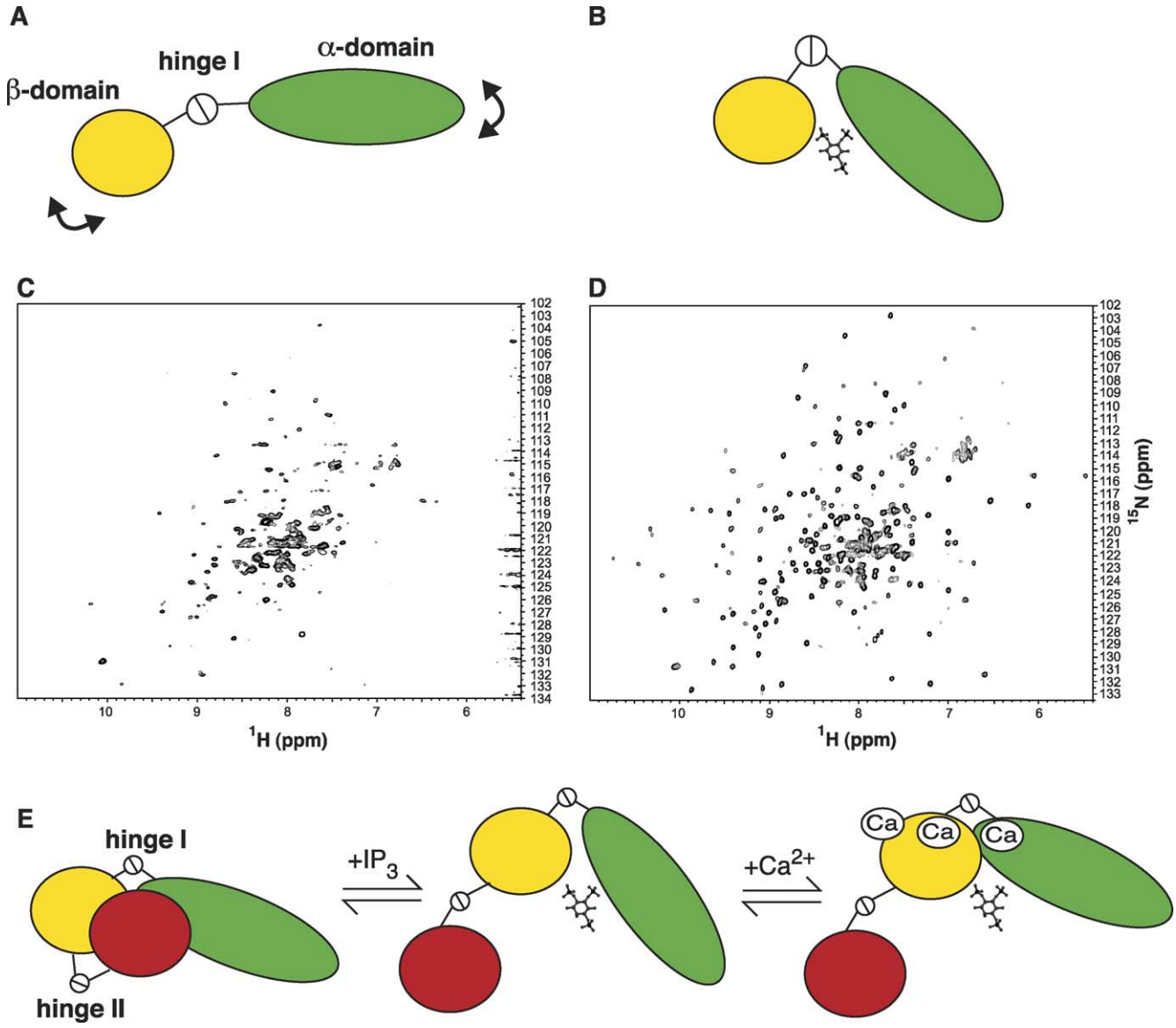

Fig. 3. IP_3 conformational restrain of IP_3R_{core}. (A) The two domains of IP_3R_{core} are shown (the β-domain in yellow and the α-domain in green) together with the hinge region between them. In the absence of the IP_3 molecule the two domains are free to move with respect to each other. (B) IP_3 binds between the two domains, thereby minimizing their motion. (C) TROSY-^{15}N, ^{1}H-HSQC of the IP_3R_{core} in Apo state suggests folded protein but quite dynamic. (D) TROSY-^{15}N, ^{1}H-HSQC of IP_3R_{core} in IP_3-bound state. Peaks are well dispersed with nearly the same intensity, suggestive of a well-folded protein with a lower range of motion than the Apo form. (E) A model for a sequence of events leading to binding of IP_3 and Ca^{2+} to the N-terminal region of the receptor. In the absence of IP_3, IP_3R_{sup} and IP_3R_{core} interact in such a way that IP_3 and Ca^{2+} binding sites are not accessible to their respected ligands. Binding of IP_3 displaces IP_3R_{sup} (red) from the IP_3 binding core β-domain (yellow) and α-domain (green), thus allowing Ca^{2+} to bind. The two hinge regions between the domains are shown. The IP_3 molecule is depicted in a stick model, and the Ca^{2+} molecules are represented by circles.

To this end, it is possible that one or both of the highly conserved surface areas found near the IP_3-binding site may serve as IP_3R1_{sup}-binding site. It is also tempting to speculate that the interaction of IP_3R1_{sup} with the IP_3R1_{core} may lead to the inhibition of Ca^{2+} binding to the IP_3R1_{core}, presumably to the Ca-II site near the IP_3 binding cleft. It is equally possible that binding of IP_3R1_{sup} to the ligand-binding site may alter the orientation of the two domains with respect to each other, thereby disrupting Ca^{2+} binding to the Ca-II site and/or Ca-I site. Taylor et al. [61] suggested that the mechanism of IP_3-binding by the receptor might be similar to that observed in ligand binding to the glutamate receptor subunits (GluR), which rigidifies domain orientation and thus leads to the activation of the channel.

In addition, several binding partners of the receptor, Ca^{2+} [62], CaM [62–64], Gβ [65] and RACK1 [43], whose binding sites reside within the first 600 residues, have been shown to effect receptor affinity for IP_3. This suggests that other cellular molecules, and possibly the region downstream from IP_3R_{core} (residues 651–1130) [21], might be involved in modulation of IP_3 binding to the receptor.

4.2. Interaction with regulatory proteins

A variety of regulatory cellular proteins of IP_3R have been shown to directly interact with IP_3R1_{sup} domain. One such protein, calmodulin (CaM), is suggested to interact with two independent segments within the first 160 residues of the receptor in a Ca^{2+}-independent manner [39]. It has been proposed that the binding of CaM to the N-terminus of IP_3R leads to IP_3-induced calcium release (IICR) inhibition [32]. Yang et al. [41] first reported that neuronal Ca^{2+}-binding protein 1 (CaBP1) binds to the first 600 residues of the receptor promoting receptor activation in the absence of the IP_3. However, several other groups later showed an antagonistic activity of CaBP1 on IP_3R channel gating [32,66,67]. Interestingly, Kasri et al. [40] reported that CaBP1 interacts with one of the CaM-binding sites. In addition, Homer proteins, which mediated interaction of IP_3R with group 1 metabotropic glutamate receptors (mGluRs), were found to associate with ^{49}PPKKF^{53} stretch on the mIP_3R1 [42]. Furthermore, a recent report revealed that RACK1, a WD40-containing scaffold protein, binds to two IP_3R segments (residues 90–110 and residues 580–600), one of which resides in IP_3R_{sup} [43]. Direct interaction between the coupling domain of the receptor (residues 2590–2749) and IP_3R_{sup} was also reported [68,69]. Clearly, the N-terminal 223 residues of IP_3 perform multiple functions, thus serving a critical role in the regulation of the channel gating.

5. Modulatory and transducing domain

The central portion of the IP_3R sequence has previously been referred to as the transducing domain, since it is involved in transferring a signal from the N-terminal ligand-binding region to the channel portion at the C-terminus of the receptor. This region is also known as the modulatory domain, since it binds a number of molecules implicated in regulation of the receptor. Small molecules, such as Ca^{2+} [23,24] and ATP [25,26], as well as proteins, such as CaM [31,32], FKBP12 [33–35], CARP [36], Gβ [65], Rack1 [43] and Caspase 3 [37], have been suggested to bind to the modulatory and transducing domain. Phosphorylation sites for PKA [27–30], PKC [70,71], PKG [72–75], CaMKKII [28,70] have also been mapped in this region. Iino, Bezprozvanny and their colleagues [76,77] recently suggested that a segment comprising residues Glu1932 to Arg2270 harbors a Ca^{2+}-sensory region and that Glu2100 is of particular importance for the Ca^{2+} sensory function. It is noteworthy that this domain shares the least homology among the three IP_3R isoforms, suggesting a regulatory variation in different subtypes. More detailed information on binding partners for this domain and their functional implications in receptor regulation has been reviewed by Patterson et al. [78].

Very little is known about the structure of the modulatory and transducing domain. Previous amino acid sequence analysis [15,60] suggested a high helical propensity and the possibility of an armadillo-like fold continuing from residue 438 to residue 1740. As we discuss in a later section, EM studies strongly suggest that this large portion of the receptor is somewhat folded into a globular structure, helping the entire tetrameric receptor form a compact shape.

6. Channel domain

Six TM helices, localized to the C-terminus of each IP_3R monomer, have been predicted. It is believed that these helices are responsible for anchoring the receptor to a membrane and creating a functional channel. In mIP_3R1 these TM regions reside between residues 2276 and 2589 with the following boundaries for each helix: TM1 2276–2294; TM2 2308–2326; TM3 2352–2372; TM4 2391–2407; TM5 2440–2462 and TM6 2570–2589 [20]. The linker segment between TM5 and TM6 is relatively long and harbors a large luminal loop (residues 2463–2528) with two N-glycosylation sites, residues Asn2475 and Asn2503 [79], as well as a pore-forming region (residues 2529–2549) [79]. This luminal segment is rich in acidic residues, which may serve to concentrate Ca^{2+} ions near the channel opening [20]. Binding of Ca^{2+} to the luminal segment was also observed [23]. Notably, IP_3R and RyR share high homology in the region encompassing TM5 and TM6 helices in IP_3R, whereas the luminal loop linking these two helices, is absent in RyR [80–83]. It is worth noting that IP_3R and RyR show negligible selectivity for Ca^{2+} over other divalent cations (Mg^{2+}, Sr^{2+} and Ba^{2+}) and are also capable of transporting monovalent cations (Na^+, K^+, Li^+, Cs^+ and Rb^+) (as reviewed in Ref. [84]). Therefore, it appears that a major reason why these channels transport Ca^{2+} stems from the high Ca^{2+} concentrations within the lumen of ER/SR.

6.1. Implications from K^+ channel structure

The exact mechanism by which IP_3R translocates Ca^{2+} from the ER is currently unknown. However, it has been suggested that the pore-forming region of IP_3R is similar to that of voltage- and second messenger-gated ion channels [79]. Williams et al. [84] proposed a mechanism based on the atomic level structure of the bacterial potassium (K^+) channel KcsA [85]. In the tetrameric structure each monomer provides two TM helices towards the formation of a functional channel with a single central pore (Fig. 4C,D,E). The structure is wider at the ion entrance and narrower at the ion exit. The outer helix in each monomer faces the lipid membrane, while the inner helix in the same monomer constitutes the central pore. The inner helices from the four subunits are all tilted with respect to the membrane, and their arrangement resembles an ‘inverted

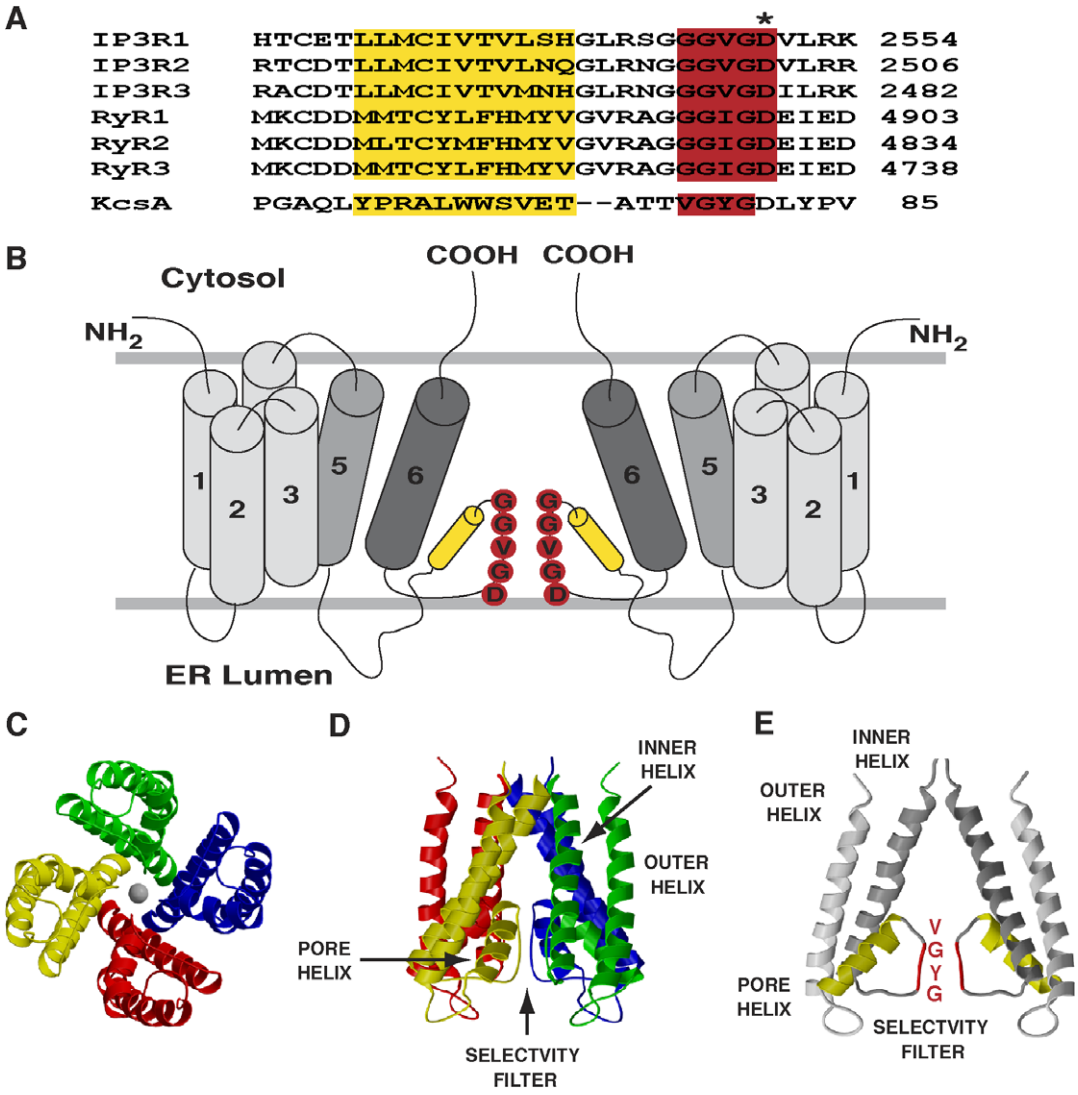

Fig. 4. Structure of the IP_3R channel domain and its pore. (A) Sequence alignment of three types of IP_3R and RyR at the region of the pore helix (highlighted in yellow) and the selectivity filter (highlighted in red). Asp2550 discussed in the text is denoted with an asterisk. Sequence of the potassium channel KcsA for the same structural segments is also shown. Alignment is based on Fig. 3 from the Shah and Sowdhamini paper [83]. (B) A model of the channel domain for the two IP_3R monomers based on the KcsA structure. The pore helix is yellow and residues comprising the selective filter are highlighted in red. Helix 5 and 6 correspond to outer and inner helices of the KcsA structure, respectively. (C) Structure of the KcsA potassium channel comprising four monomers (green, blue, red and yellow) viewed from the extracellular side. (D) Side view of KcsA channel. (E) The side view of the two monomers of KcsA with outer helix (light gray), inner helix (dark gray), pore helix (yellow) and the selectivity filter (red) shown. GenBank accession nos. mouse IP_3R1, X15373; human IP_3R2, D26350; human IP_3R3, D26351; rabbit RyR1, X15209; rabbit RyR2, U50465; rabbit RyR3, X68650; *S. lividans* KcsA, AL939132.

teepee' [85]. It is the segment linking the two helices that contains a pore-forming region (helical in nature) and a selectivity filter (^{77}VGYG) (Fig. 4E). It has been proposed that the TM5 and TM6 of IP_3R, and the corresponding helices in RyR, correlate with the inner and outer helices of each KcsA monomer [82,84] (Fig. 4B). The proposed pore-forming segment [79] and selectivity filter (^{2546}GGVGD in mIP_3R1) [86,87] are conserved among these ion channels (Fig. 4A), and most likely reside close to the luminal side of a membrane (Fig. 4B). Interestingly, mutations of Asp2550, which serves as one of the key residues in selectivity filter, to Ala or Asn resulted in channel impermeability to Ca^{2+} ions [87]. Moreover, substitution of Asp2550 with Glu produced channels lacking selectivity for Ca^{2+} over K^+ [88]. From these analyses it has been proposed that Asp2550 functions to concentrate Ca^{2+} ions at the entrance of the central pore and to discriminate between Ca^{2+} and K^+ [87,88]. It has also been shown that Val2548 is critical for ion conductance control, since its mutation to Ile lead to channels with higher K^+ permeation but the same selectivity as the wild-type [88].

Recently acquired structural information on bacterial potassium channels uncovered the topological arrangement of the central pore and defined the underlying gating principles [85,89–96]. Structural comparison between IP_3Rs and other ion channels may provide glimpses into the

overall arrangement of the channel domain of IP_3R. However, determination of the precise mechanisms of IP_3R channel gating requires structural elucidation of not only the channel domain, but of the entire molecule, as IP_3R possesses a large cytoplasmic region with channel modulatory functions which are not present in K^+ channels.

7. Coupling domain

An even number of TM helices indicates that the C-terminal tail of the receptor resides in the cytoplasm (2590–2749). At present very little is known about the structure and function of the last 160 residues of IP_3R. Secondary structure prediction of this portion of the receptor suggests that the region is highly helical in nature. A direct interaction of the C-terminal domain with the N-terminus of the receptor has been reported [68,97], providing a compelling mechanism for the action of this region. From these observations this region became known as the coupling domain. The coupling domain is essential for the formation of the functional Ca^{2+} channel since binding of monoclonal antibody to the residues 2736–2747 [98], and the expression of the C-terminal GFP-fused IP_3R [99], produce dysfunctional IICR.

7.1. Role of the coupling domain in receptor oligomerization

Formation of the functional tetrameric channel requires both the TM region and the coupling domain [18], whereas the first TM helix appears to be sufficient for receptor anchoring to the membrane [100]. Extensive deletion mutagenesis of the C-terminal portion of the receptor defined TM5 and TM6 as more important for intermolecular interactions than the preceding TM helices [81,100]. The C-terminal residues 2629–2654, within the coupling domain, have been shown to be the minimal segment required for the dimerization of this domain in vitro [101]. The importance of TM5 and TM6, as well as the coupling domain in the formation of a functional channel, is supported by the observation that deletion of the TM1–4 produces a channel with permeation properties similar to the native channel [82].

7.2. Head-to-tail interaction in the receptor

Limited trypsinization studies of the full-length receptor produced five major fragments (Fig. 1A) capable of associating with each other through non-covalent interactions in order to form the functional channel. Direct interaction was detected between the N- and C-termini of IP_3R [68,97]. Moreover, cross-linking and pull-down assays defined residues 1–605 from the N-terminus and residues 2418–2749 from the C-terminus as the interaction sites, with association occurring in a inter-subunit manner [69]. In addition to the coupling domain, TM5 and TM6 have been considered to play a role in the head-to-tail interaction. The importance of the coupling domain is highlighted by the observation that removal of only the last 14 residues (2736–2749) caused a decrease in the Ca^{2+} conductance of the receptor [21]. Furthermore, mutation of two highly conserved Cys residues (C2610, C2613) within the coupling domain resulted in a non-functional Ca^{2+}-channel. At the N-terminus, residues 1–223 were found to be most critical for functional coupling between the N- and C-terminal regions of the receptor [21]. Similar head-to-tail interaction was also observed in the cyclic nucleotide-gated ion channels [102–104] and the voltage-gated potassium channels [105,106]. In those channels, a disulfide bond formation between N- and C-terminal domains was shown to be responsible for the interaction [103–105]. Formation of such disulfide bonds involving C2610 and C2613 in IP_3R remains to be determined.

8. Functional tetrameric structure

Obtaining structural information on full-length IP_3R has proven to be challenging. Its large molecular size (~300 kDa), in addition to its membrane association, presents a major hurdle in 3D structure determination. Furthermore, the inability to obtain a large amount of functional tetrameric protein by recombinant techniques has hampered X-ray crystallography, as well as solution and solid-state NMR studies. Consequently, all structural information available to date on the entire receptor is based on EM studies of the immunoaffinity-purified protein from high-content tissues such as cerebellum. Previous 2D negative staining [107–109], or 2D crystalline array EM studies [110,111], revealed a rough estimate on the receptor size and shape. More recently, five 3D structures of IP_3R1 were reconstructed using single particle EM analysis at resolutions of 34–20 Å. All structures identified a 4-fold symmetry within the channel, consisting of two morphologically distinctive regions: a cytoplasmic domain and a channel domain. However, interpretations of the reconstructed structures, obtained in four different laboratories, differ from one another. Below, we summarize the findings from the EM studies.

8.1. Variation in 3D reconstruction of IP_3R in IP_3- and Ca^{2+}-free state

The 3D structure of immunoaffinity purified IP_3R1 by Serysheva et al. [13] showed an overall shape with dimensions of 250×250×165 $Å^3$ possessing a ‘pinwheel’ appearance with a central square-shaped domain and four ‘curved radial spikes’ protruding from it. The two large domains make up the spike and the other two reside in the core region together with the TM domain. The previously determined crystal structure of IP_3R_{core} was fitted into one

of the spike-domains. According to their reconstructed model [13], IP_3R_{sup} was proposed to be in close proximity to the C-terminal coupling domain, which resides at the center of the pinwheel structure.

The single particle EM analysis of a cerebellar-purified IP_3R1 by da Fonseca et al. [12] revealed a structure with a 'flower-like' appearance and dimensions of 180×180×180 $Å^3$. The two large domains, described as the 'petal' and the 'stigma' of a flower, were proposed to correspond to the cytoplasmic portion of the receptor. The smaller domain, resembling the stalk of a flower, was assigned to the channel domain. Slender connections were observed between the two cytoplasmic domains and between one of the cytoplasmic domains and the channel domain. In this EM model, IP_3R_{core} was positioned at the interior of the structure (i.e. within the stigma portion). The channel domain of the structure accounted for about 35% of its electron density, considerably in excess of what the six TM segments and their connecting loops should occupy. This suggests that the domain contains not only the TM segment but also part of the modulatory domain and coupling domain [12].

Jiang et al. [10] reported a relatively compact structure of cerebellar purified IP_3R1 with overall dimensions of 180×180×170 $Å^3$. The structure appeared to resemble a 'propeller' with a majority of its density comprising the central core region and the rest making up the four protruding blades. The central core region was shown to consist of two asymmetrical domains with the smaller portion possibly representing the TM region of the receptor, and the larger portion corresponding to the cytosolic region [10]. The function of the protruding parts is not known at this time, but it may represent an IP_3-binding core.

The highest resolution EM structure of IP_3R1 from cerebellum, determined by Sato et al. [14], revealed a structure reminiscent of a 'hot air balloon'. The balloon portion (diameter 175 Å) was assigned to the cytoplasmic domain with the square-shaped basket portion (side length of 96 Å) assigned to the channel domain (Fig. 5A). Within the cytoplasmic domain, internal cavities were observed and a prominent L-shaped density was present at the top part of the molecule, shown to correspond to IP_3R_{core}. A 3D modeling of the crystal structure of IP_3R_{core} to this L-shaped density required bending of the IP_3R_{core} structure at the linker between the α- and β-domains, suggesting that IP_3-binding induces a conformational change within IP_3R_{core} [14] (Fig. 5B). The unaccounted density around the linker between the two domains is assigned to IP_3R_{sup} [14]. It is interesting to note that this portion of the L-shaped density is in direct contact with the inner tubular density of the receptor. This may correspond to head-to-tail interaction that was discovered by previous biochemical work [68,69,97].

8.2. Ca^{2+}-induced structural changes

Hamada et al. [11] examined the effect of Ca^{2+} on the 3-D EM structure of the tetrameric IP_3R channel. In the absence of Ca^{2+} the structure had a mushroom-like appearance with overall dimensions of 190×190×160 $Å^3$ (Fig. 5C). The cytoplasmic portion of the receptor comprises a large square-shaped head connected to a channel domain via four bridges. In the presence of Ca^{2+} the structure resembles a 'windmill'-shape with an elliptical cytoplasmic domain (93×91×72 $Å^3$) protruding away from the channel domain, which was assigned to the central core structure (85×85×100 $Å^3$). This conformational transition between closed (Ca^{2+}-free) and open (Ca^{2+}-bound) states probably represents a functional change associated with high affinity Ca^{2+} binding ($<10^{-7}$ M) [11]. Interestingly, cross-sections of the two structures showed a less defined boundary of the channel domain in the 'open form' than in the 'closed form', suggesting a significant Ca^{2+}-induced conformational change within the channel domain. Considering that these 3D structures were solved in the absence of IP_3, Hamada et al. [11] cannot exclude the possibility that the windmill structure may represent a "desensitized" state of the receptor, resulting from the prolonged exposure to Ca^{2+}. The combination of heparin-gold labeling and 2D imaging of the receptor, with electron density fitting in a 3D reconstruction, led to the proposal that the IP_3R_{core} was located at the peripheral region of the mushroom structure and within the protruding sails of the windmill structure (Fig. 5C).

Despite inconsistencies in the overall appearance of these reconstructed 3D structures of IP_3R1, EM studies have nevertheless provided insights into the gross facade of the receptor. Variations in the overall appearance of these structures could arise from differences in sample preparation, sample heterogeneity and method employed for the single particle image analysis in the 3D reconstruction. Resolution of IP_3R structures will improve with time, and we will be able to resolve the detailed architecture of the functional channel from structural studies. Furthermore, site-specific labeling and modeling of atomic resolution structures of individual domains of IP_3R into the EM-derived structure of the entire receptor will further our understanding of the structure–function relationship of IP_3Rs.

Considering all the current biochemical and structural information described above, we propose that Ca-I and/or Ca-II sites correspond to "high affinity" Ca^{2+}-binding sites that possess a stimulatory function in channel opening [112]. The inhibitory "low affinity" sites [113] likely reside in the modulatory domain or Ca^{2+}-binding proteins (CaM, CaBP1). In addition to Ca-I and Ca-II sites, other Ca^{2+}-binding sites in the modulatory domain (in particular Glu2100 in a proposed Ca^{2+}-sensory region) may also exhibit a stimulatory function [76,77]. In a two-step model for channel activation (Fig. 5D), the first step involves IP_3 binding which mainly causes a localized conformational change at the N-terminal region, as seen in the X-ray crystallographic studies [15], and possibly in its neighboring regions. This IP_3-induced structural change may result in activation of some Ca^{2+}-binding

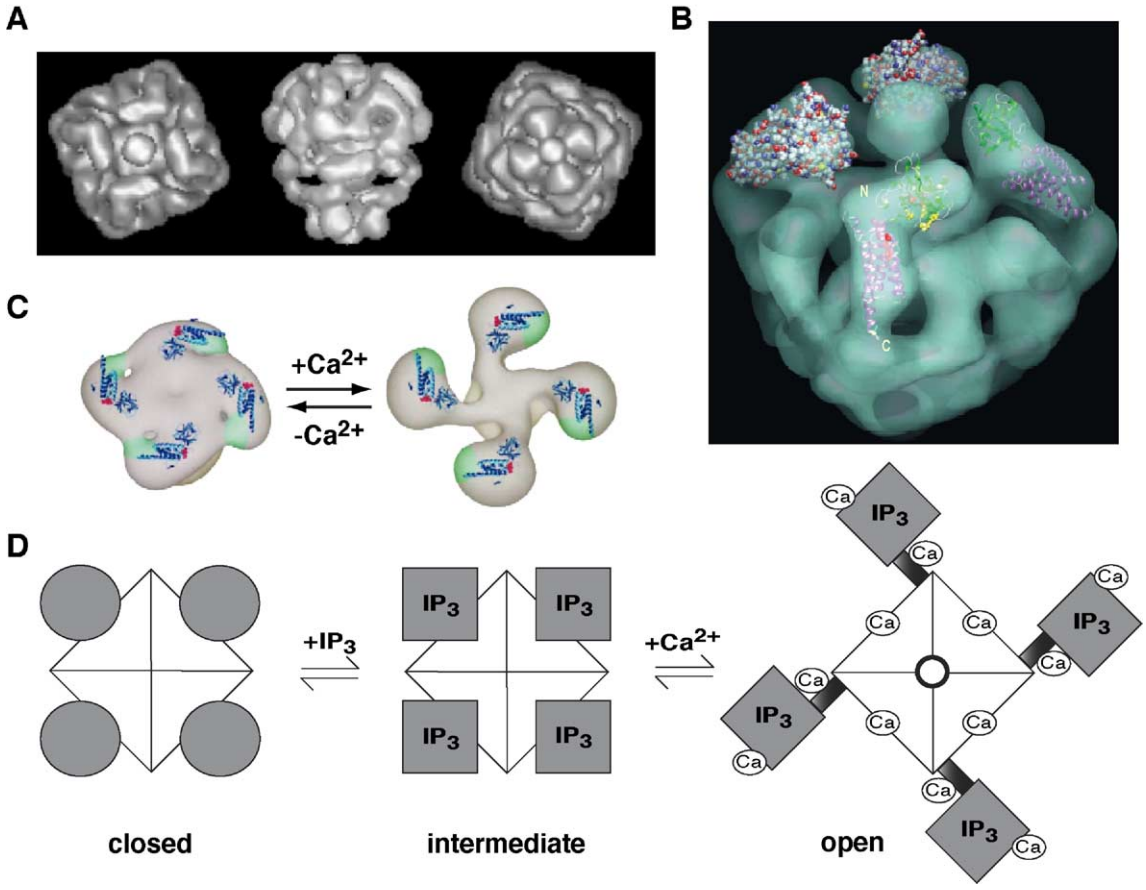

Fig. 5. EM derived 3D reconstructions of IP_3R1. (A) Highest resolution structure of IP_3R1, determined in the absence of IP_3 and Ca^{2+}, and viewed from the cytosol, side and ER lumen, respectively (adapted from Sato et al. [14], Figs. 2A, 3A and 2F, respectively). (B) Modeling of IP_3R_{core}, in the IP_3-free state (ribbon and space filled model), into the density map of the whole receptor (adapted from Sato et al. [14], Fig. 4F). (C) Global structural change of IP_3R1 resulting from the presence of Ca^{2+}. The structure of IP_3R_{core} (blue) in complex with IP_3 (red) is modeled in both conformational states (adapted from Hamada et al. [11], Fig. 9B). (D) A possible three-state model for receptor activation. Symmetrical structure representing four receptor monomers, which comprise the functional channel. Shaded circles represent the ligand-binding domain in the receptor. The IP_3-induced conformational change in the ligand binding domain of the 'closed' state (depicted in Fig. 3E) is represented as a circle to square transition, leading to the 'intermediate' state. This first step conformational change may be localized within the ligand binding and its neighboring region, leading to Ca^{2+} binding to a number of different sites across the receptor. Some of these sites may only become functional following IP_3 binding. The second step conformational change, induced by Ca^{2+} binding, results in a global structural change involving not only the ligand binding but also the modulatory and channel domains in the tetrameric receptor (see text for more details). Note that the "intermediate" state may represent multiple conformational states due to Ca^{2+}-binding to many different sites in the receptor.

sites [15]. The second step of the process is initiated by Ca^{2+}-binding to multiple sites on the receptor, which likely induces a more drastic conformational change in the channel, as evidenced by EM studies [11,109]. This Ca^{2+}-induced conformational change causes the displacement of the IP_3-binding region from the center of the channel, which leads to channel opening (Fig. 5D). The consortium of IP_3 and Ca^{2+} in receptor activation ensures accuracy of the gating function and amplification of the output signal in response to an external stimulus. Previous studies [76,77], however, suggested that the Ca^{2+}-sensory region within the modulatory domain also functions in the inhibition of channel gating under certain conditions. Although accurate understanding of this complex regulatory mechanism of the channel requires elucidation of the exact function of each Ca^{2+}-binding site found on the receptor, our model as well as the models proposed previously [5,114], should serve as working hypotheses for researchers in the field.

9. Conclusion and future prospective

Since the discovery of IP_3R as a glycoprotein in the cerebellum [115], a great deal has been learned about its function and structure. Regulation of IP_3R function as a Ca^{2+} release channel involves two important second messengers, IP_3 and Ca^{2+}. We now know the atomic-resolution structure of the IP_3 binding domain of the receptor in complex with IP_3, and are beginning to understand how the functional tetrameric channel responds to the two important cellular ligands. The IP_3 molecule serves as the first key in the multi-lock IP_3R system, with full channel activation observed only after the 'insertion' of the second

key, Ca^{2+}. Calcium binding proteins (CaM and CaBP1) interact with IP_3R and promote channel closure when the cytoplasmic Ca^{2+} levels are too high. Therefore, Ca^{2+} acts as both a positive and negative channel regulator. Understanding the exact mechanism needed to unlock and then lock the channel is a challenging task for structural biologists. Numerous cellular interacting partners of IP_3R have recently been found to modulate the channel function, with more likely to be discovered in the future. Despite the recent advances made by X-ray and EM studies on the IP_3R structure–function relationship, many fundamental questions still remain unanswered. From a structural biology perspective, we will need to determine the atomic-resolution structures of various parts of the molecule, including the suppressor domain, the modulatory and transducing domain, the channel domain, and the coupling domain. We also need to better define how Ca^{2+} ions interact with the receptor to induce channel activation. Moreover, elucidation of the structural basis for receptor interaction with various binding partners such as CaM, CaBP1, among others, is required. It is also important to decipher the exact mechanism of ion translocation within the channel domain. Finally, posttranslational modifications, such as phosphorylation and glycosylation, will add another dimension to the already complex regulatory mechanisms of IP_3R.

Acknowledgements

This work was supported by a fellowship from the Canadian Institute of Health and Research to I.B., by a grant from the Heart and Stroke Foundation of Canada to M.I. and by grants from the Ministry of Education, Science, Sports, and Culture of Japan to M.K. We would like to thank Jane Gooding, Brigitte Maurer, Jenny Chan and Tapas Kumar Mal for critical review of the manuscript. M.I. is a Canada Research Chair in Cancer Structural Biology.

References

[1] M.J. Berridge, Inositol trisphosphate and calcium signalling, Nature 361 (1993) 315–325.
[2] M.J. Berridge, P. Lipp, M.D. Bootman, The versatility and universality of calcium signalling, Nat. Rev., Mol. Cell Biol. 1 (2000) 11–21.
[3] M.J. Berridge, M.D. Bootman, H.L. Roderick, Calcium signalling: dynamics, homeostasis and remodelling, Nat. Rev., Mol. Cell Biol. 4 (2003) 517–529.
[4] N. Nadif Kasri, G. Bultynck, I. Sienaert, G. Callewaert, C. Erneux, L. Missiaen, J.B. Parys, H. De Smedt, The role of calmodulin for inositol 1,4,5-trisphosphate receptor function, Biochim. Biophys. Acta (BBA)-Proteins Proteomics 1600 (2002) 19–31.
[5] C.W. Taylor, A.J. Laude, IP_3 receptors and their regulation by calmodulin and cytosolic Ca^{2+}, Cell Calcium 32 (2002) 321–334.
[6] T. Furuichi, K. Kohda, A. Miyawaki, K. Mikoshiba, Intracellular channels, Curr. Opin. Neurobiol. 4 (1994) 294–303.
[7] S. Patel, S.K. Joseph, A.P. Thomas, Molecular properties of inositol 1,4,5-trisphosphate receptors, Cell Calcium 25 (1999) 247–264.
[8] T. Furuichi, D. Simon-Chazottes, I. Fujino, N. Yamada, M. Hasegawa, A. Miyawaki, S. Yoshikawa, J.L. Guenet, K. Mikoshiba, Widespread expression of inositol 1,4,5-trisphosphate receptor type 1 gene ($InsP_3R1$) in the mouse central nervous system, Receptors Channels 1 (1993) 11–24.
[9] P.F. Worley, J.M. Baraban, J.S. Colvin, S.H. Snyder, Inositol trisphosphate receptor localization in brain: variable stoichiometry with protein kinase C, Nature 325 (1987) 159–161.
[10] Q.X. Jiang, E.C. Thrower, D.W. Chester, B.E. Ehrlich, F.J. Sigworth, Three-dimensional structure of the type 1 inositol 1,4,5-trisphosphate receptor at 24 Å resolution, EMBO J. 21 (2002) 3575–3581.
[11] K. Hamada, A. Terauchi, K. Mikoshiba, Three-dimensional rearrangements within inositol 1,4,5-trisphosphate receptor by calcium, J. Biol. Chem. 278 (2003) 52881–52889.
[12] P.C. da Fonseca, S.A. Morris, E.P. Nerou, C.W. Taylor, E.P. Morris, Domain organization of the type 1 inositol 1,4,5-trisphosphate receptor as revealed by single-particle analysis, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 3936–3941.
[13] I. Serysheva II, D.J. Bare, S.J. Ludtke, C.S. Kettlun, W. Chiu, G.A. Mignery, Structure of the type 1 inositol 1,4,5-trisphosphate receptor revealed by electron cryomicroscopy, J. Biol. Chem. 278 (2003) 21319–21322.
[14] C. Sato, K. Hamada, T. Ogura, A. Miyazawa, K. Iwasaki, Y. Hiroaki, K. Tani, A. Terauchi, Y. Fujiyoshi, K. Mikoshiba, Inositol 1,4,5-trisphosphate receptor contains multiple cavities and L-shaped ligand-binding domains, J. Mol. Biol. 336 (2004) 155–164.
[15] I. Bosanac, J.R. Alattia, T.K. Mal, J. Chan, S. Talarico, F.K. Tong, K.I. Tong, F. Yoshikawa, T. Furuichi, M. Iwai, T. Michikawa, K. Mikoshiba, M. Ikura, Structure of the inositol 1,4,5-trisphosphate receptor binding core in complex with its ligand, Nature 420 (2002) 696–700.
[16] T. Furuichi, S. Yoshikawa, A. Miyawaki, K. Wada, N. Maeda, K. Mikoshiba, Primary structure and functional expression of the inositol 1,4,5-trisphosphate-binding protein P400, Nature 342 (1989) 32–38.
[17] G.A. Mignery, T.C. Sudhof, The ligand binding site and transduction mechanism in the inositol-1,4,5-triphosphate receptor, EMBO J. 9 (1990) 3893–3898.
[18] A. Miyawaki, T. Furuichi, Y. Ryou, S. Yoshikawa, T. Nakagawa, T. Saitoh, K. Mikoshiba, Structure–function relationships of the mouse inositol 1,4,5-trisphosphate receptor, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 4911–4915.
[19] F. Yoshikawa, M. Morita, T. Monkawa, T. Michikawa, T. Furuichi, K. Mikoshiba, Mutational analysis of the ligand binding site of the inositol 1,4,5-trisphosphate receptor, J. Biol. Chem. 271 (1996) 18277–18284.
[20] S. Yoshikawa, T. Tanimura, A. Miyawaki, M. Nakamura, M. Yuzaki, T. Furuichi, K. Mikoshiba, Molecular cloning and characterization of the inositol 1,4,5-trisphosphate receptor in *Drosophila melanogaster*, J. Biol. Chem. 267 (1992) 16613–16619.
[21] K. Uchida, H. Miyauchi, T. Furuichi, T. Michikawa, K. Mikoshiba, Critical regions for activation gating of the inositol 1,4,5-trisphosphate receptor, J. Biol. Chem. 278 (2003) 16551–16560.
[22] G.A. Mignery, P.A. Johnston, T.C. Sudhof, Mechanism of Ca^{2+} inhibition of inositol 1,4,5-trisphosphate ($InsP_3$) binding to the cerebellar $InsP_3$ receptor, J. Biol. Chem. 267 (1992) 7450–7455.
[23] I. Sienaert, H. De Smedt, J.B. Parys, L. Missiaen, S. Vanlingen, H. Sipma, R. Casteels, Characterization of a cytosolic and a luminal Ca^{2+} binding site in the type I inositol 1,4,5-trisphosphate receptor, J. Biol. Chem. 271 (1996) 27005–27012.
[24] I. Sienaert, L. Missiaen, H. De Smedt, J.B. Parys, H. Sipma, R. Casteels, Molecular and functional evidence for multiple Ca^{2+}-binding domains in the type 1 inositol 1,4,5-trisphosphate receptor, J. Biol. Chem. 272 (1997) 25899–25906.
[25] C.D. Ferris, R.L. Huganir, S.H. Snyder, 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. 87 (1990) 2147–2151.
[26] N. Maeda, T. Kawasaki, S. Nakade, N. Yokota, T. Taguchi, M. Kasai, K. Mikoshiba, Structural and functional characterization of inositol 1,4,5-trisphosphate receptor channel from mouse cerebellum, J. Biol. Chem. 266 (1991) 1109–1116.
[27] S. Supattapone, S.K. Danoff, A. Theibert, S.K. Joseph, J. Steiner, S.H. Snyder, Cyclic AMP-dependent phosphorylation of a brain inositol trisphosphate receptor decreases its release of calcium, Proc. Natl. Acad. Sci. U. S. A. 85 (1988) 8747–8750.
[28] H. Yamamoto, N. Maeda, M. Niinobe, E. Miyamoto, K. Mikoshiba, Phosphorylation of P400 protein by cyclic AMP-dependent protein kinase and Ca^{2+}/calmodulin-dependent protein kinase II, J. Neurochem. 53 (1989) 917–923.
[29] S.K. Danoff, C.D. Ferris, C. Donath, G.A. Fischer, S. Munemitsu, A. Ullrich, S.H. Snyder, C.A. Ross, 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. 88 (1991) 2951–2955.
[30] C.D. Ferris, A.M. Cameron, D.S. Bredt, R.L. Huganir, S.H. Snyder, Inositol 1,4,5-trisphosphate receptor is phosphorylated by cyclic AMP-dependent protein kinase at serines 1755 and 1589, Biochem. Biophys. Res. Commun. 175 (1991) 192–198.
[31] M. Yamada, A. Miyawaki, K. Saito, T. Nakajima, M. Yamamoto-Hino, Y. Ryo, T. Furuichi, K. Mikoshiba, The calmodulin-binding domain in the mouse type 1 inositol 1,4,5-trisphosphate receptor, Biochem. J. 308 (Pt. 1) (1995) 83–88.
[32] N.N. Kasri, G. Bultynck, J. Smyth, K. Szlufcik, J.B. Parys, G. Callewaert, L. Missiaen, R.A. Fissore, K. Mikoshiba, H. de Smedt, The N-terminal Ca^{2+}-independent calmodulin-binding site on the inositol 1,4,5-trisphosphate receptor is responsible for calmodulin inhibition, even though this inhibition requires Ca^{2+}, Mol. Pharmacol. 66 (2004) 276–284.
[33] A.M. Cameron, J.P. Steiner, D.M. Sabatini, A.I. Kaplin, L.D. Walensky, S.H. Snyder, Immunophilin FK506 binding protein associated with inositol 1,4,5-trisphosphate receptor modulates calcium flux, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 1784–1788.
[34] A.M. Cameron, J.P. Steiner, A.J. Roskams, S.M. Ali, G.V. Ronnett, S.H. Snyder, Calcineurin associated with the inositol 1,4,5-trisphosphate receptor-FKBP12 complex modulates Ca^{2+} flux, Cell 83 (1995) 463–472.
[35] A.M. Cameron, F.C. Nucifora Jr., E.T. Fung, D.J. Livingston, R.A. Aldape, C.A. Ross, S.H. Snyder, FKBP12 binds the inositol 1,4,5-trisphosphate receptor at leucine-proline (1400–1401) and anchors calcineurin to this FK506-like domain, J. Biol. Chem. 272 (1997) 27582–27588.
[36] J. Hirota, H. Ando, K. Hamada, K. Mikoshiba, Carbonic anhydrase-related protein is a novel binding protein for inositol 1,4,5-trisphosphate receptor type 1, Biochem. J. 372 (2003) 435–441.
[37] J. Hirota, T. Furuichi, K. Mikoshiba, Inositol 1,4,5-trisphosphate receptor type 1 is a substrate for caspase-3 and is cleaved during apoptosis in a caspase-3-dependent manner, J. Biol. Chem. 274 (1999) 34433–34437.
[38] C.E. Adkins, S.A. Morris, H. De Smedt, I. Sienaert, K. Torok, C.W. Taylor, Ca^{2+}-calmodulin inhibits Ca^{2+} release mediated by type-1, -2 and -3 inositol trisphosphate receptors, Biochem. J. 345 (Pt. 2) (2000) 357–363.
[39] I. Sienaert, N. Nadif Kasri, S. Vanlingen, J.B. Parys, G. Callewaert, L. Missiaen, H. De Smedt, Localization and function of a calmodulin/apocalmodulin binding domain in the N-terminal part of the type 1 inositol 1,4,5-trisphosphate receptor, Biochem. J. 365 (2002) 269–277.
[40] N.N. Kasri, A.M. Holmes, G. Bultynck, J.B. Parys, M.D. Bootman, K. Rietdorf, L. Missiaen, F. McDonald, H.D. Smedt, S.J. Conway, A.B. Holmes, M.J. Berridge, H.L. Roderick, Regulation of $InsP_3$ receptor activity by neuronal Ca^{2+}-binding proteins, EMBO J. 23 (2004) 312–321.
[41] J. Yang, S. McBride, D.O. Mak, N. Vardi, K. Palczewski, F. Haeseleer, J.K. Foskett, Identification of a family of calcium sensors as protein ligands of inositol trisphosphate receptor Ca^{2+} release channels, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 7711–7716.
[42] J.C. Tu, B. Xiao, J.P. Yuan, A.A. Lanahan, K. Leoffert, M. Li, D.J. Linden, P.F. Worley, Homer binds a novel proline-rich motif and links group 1 metabotropic glutamate receptors with IP_3 receptors, Neuron 21 (1998) 717–726.
[43] R.L. Patterson, D.B. van Rossum, R.K. Barrow, S.H. Snyder, RACK1 binds to inositol 1,4,5-trisphosphate receptors and mediates Ca^{2+} release, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 2328–2332.
[44] S.H. Yoo, M.S. Lewis, Thermodynamic study of the pH-dependent interaction of chromogranin A with an intraluminal loop peptide of the inositol 1,4,5-trisphosphate receptor, Biochemistry 34 (1995) 632–638.
[45] S.H. Yoo, M.S. Lewis, Interaction between an intraluminal loop peptide of the inositol 1,4,5-trisphosphate receptor and the near N-terminal peptide of chromogranin A, FEBS Lett. 427 (1998) 55–58.
[46] L.Y. Bourguignon, H. Jin, Identification of the ankyrin-binding domain of the mouse T-lymphoma cell inositol 1,4,5-trisphosphate (IP_3) receptor and its role in the regulation of IP_3-mediated internal Ca^{2+} release, J. Biol. Chem. 270 (1995) 7257–7260.
[47] D. Boehning, R.L. Patterson, L. Sedaghat, N.O. Glebova, T. Kurosaki, S.H. Snyder, Cytochrome *c* binds to inositol (1,4,5) trisphosphate receptors, amplifying calcium-dependent apoptosis, Nat. Cell Biol. 5 (2003) 1051–1061.
[48] S. Zhang, A. Mizutani, C. Hisatsune, T. Higo, H. Bannai, T. Nakayama, M. Hattori, K. Mikoshiba, Protein 4.1 N is required for translocation of inositol 1,4,5-trisphosphate receptor type 1 to the basolateral membrane domain in polarized Madin–Darby canine kidney cells, J. Biol. Chem. 278 (2003) 4048–4056.
[49] A. Maximov, T.S. Tang, I. Bezprozvanny, Association of the type 1 inositol (1,4,5)-trisphosphate receptor with 4.1 N protein in neurons, Mol. Cell. Neurosci. 22 (2003) 271–283.
[50] J. Cui, S.J. Matkovich, N. deSouza, S. Li, N. Rosemblit, A.R. Marks, Regulation of the type 1 inositol 1,4,5-trisphosphate receptor by phosphorylation at tyrosine 353, J. Biol. Chem. 279 (2004) 16311–16316.
[51] H. Ando, A. Mizutani, T. Matsu-ura, K. Mikoshiba, IRBIT, a novel inositol 1,4,5-trisphosphate (IP_3) receptor-binding protein, is released from the IP_3 receptor upon IP_3 binding to the receptor, J. Biol. Chem. 278 (2003) 10602–10612.
[52] R.J. Mourey, V.A. Estevez, J.F. Marecek, R.K. Barrow, G.D. Prestwich, S.H. Snyder, Inositol 1,4,5-trisphosphate receptors: labeling the inositol 1,4,5-trisphosphate binding site with photoaffinity ligands, Biochemistry 32 (1993) 1719–1726.
[53] R.A. Wilcox, W.U. Primrose, S.R. Nahorski, R.A. Challiss, New developments in the molecular pharmacology of the myo-inositol 1,4,5-trisphosphate receptor, Trends Pharmacol. Sci. 19 (1998) 467–475.
[54] A.M. Riley, S.A. Morris, E.P. Nerou, V. Correa, B.V. Potter, C.W. Taylor, Interactions of inositol 1,4,5-trisphosphate (IP_3) receptors with synthetic poly(ethylene glycol)-linked dimers of IP_3 suggest close spacing of the IP_3-binding sites, J. Biol. Chem. 277 (2002) 40290–40295.
[55] A.M. Riley, A.J. Laude, C.W. Taylor, B.V. Potter, Dimers of D-myo-inositol 1,4,5-trisphosphate: design, synthesis, and interaction with $Ins(1,4,5)P_3$ receptors, Bioconjug. Chem. 15 (2004) 278–289.
[56] S.R. Nahorski, B.V. Potter, Molecular recognition of inositol polyphosphates by intracellular receptors and metabolic enzymes, Trends Pharmacol. Sci. 10 (1989) 139–144.
[57] R.A. Wilcox, C. Erneux, W.U. Primrose, R. Gigg, S.R. Nahorski, 2-Hydroxyethyl-alpha-D-glucopyranoside-2,3′,4′-trisphosphate, a novel, metabolically resistant, adenophostin A and myo-inositol-1,4,5-trisphosphate analogue, potently interacts with the myo-inositol-1,4,5-trisphosphate receptor, Mol. Pharmacol. 47 (1995) 1204–1211.

[58] H.J. Rosenberg, A.M. Riley, A.J. Laude, C.W. Taylor, B.V. Potter, Synthesis and Ca^{2+}-mobilizing activity of purine-modified mimics of adenophostin A: a model for the adenophostin-Ins(1,4,5)P_3 receptor interaction, J. Med. Chem. 46 (2003) 4860–4871.
[59] F. Yoshikawa, T. Uchiyama, H. Iwasaki, C. Tomomori-Satoh, T. Tanaka, T. Furuichi, K. Mikoshiba, High efficient expression of the functional ligand binding site of the inositol 1,4,5-triphosphate receptor in *Escherichia coli*, Biochem. Biophys. Res. Commun. 257 (1999) 792–797.
[60] C.P. Ponting, Novel repeats in ryanodine and IP_3 receptors and protein *O*-mannosyltransferases, Trends Biochem. Sci. 25 (2000) 48–50.
[61] C.W. Taylor, P.C. da Fonseca, E.P. Morris, IP_3 receptors: the search for structure, Trends Biochem. Sci. 29 (2004) 210–219.
[62] H. Sipma, P. De Smet, I. Sienaert, S. Vanlingen, L. Missiaen, J.B. Parys, H. De Smedt, Modulation of inositol 1,4,5-trisphosphate binding to the recombinant ligand-binding site of the type-1 inositol 1,4, 5-trisphosphate receptor by Ca^{2+} and calmodulin, J. Biol. Chem. 274 (1999) 12157–12162.
[63] S. Patel, S.A. Morris, C.E. Adkins, G. O'Beirne, C.W. Taylor, Ca^{2+}-independent inhibition of inositol trisphosphate receptors by calmodulin: redistribution of calmodulin as a possible means of regulating Ca^{2+} mobilization, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 11627–11632.
[64] T.J. Cardy, C.W. Taylor, A novel role for calmodulin: Ca^{2+}-independent inhibition of type-1 inositol trisphosphate receptors, Biochem. J. 334 (Pt. 2) (1998) 447–455.
[65] W. Zeng, D.O. Mak, Q. Li, D.M. Shin, J.K. Foskett, S. Muallem, A new mode of Ca^{2+} signaling by G protein-coupled receptors: gating of IP_3 receptor Ca^{2+} release channels by Gbetagamma, Curr. Biol. 13 (2003) 872–876.
[66] L.P. Haynes, A.V. Tepikin, R.D. Burgoyne, Calcium-binding protein 1 is an inhibitor of agonist-evoked, inositol 1,4,5-trisphosphate-mediated calcium signaling, J. Biol. Chem. 279 (2004) 547–555.
[67] N.N. Kasri, I. Sienaert, J.B. Parys, G. Callewaert, L. Missiaen, A. Jeromin, H. De Smedt, A novel Ca^{2+}-induced Ca^{2+} release mechanism in A7r5 cells regulated by calmodulin-like proteins, J. Biol. Chem. 278 (2003) 27548–27555.
[68] F. Yoshikawa, H. Iwasaki, T. Michikawa, T. Furuichi, K. Mikoshiba, Trypsinized cerebellar inositol 1,4,5-trisphosphate receptor. Structural and functional coupling of cleaved ligand binding and channel domains, J. Biol. Chem. 274 (1999) 316–327.
[69] D. Boehning, S.K. Joseph, Direct association of ligand-binding and pore domains in homo- and heterotetrameric inositol 1,4,5-trisphosphate receptors, EMBO J. 19 (2000) 5450–5459.
[70] C.D. Ferris, R.L. Huganir, D.S. Bredt, A.M. Cameron, S.H. Snyder, Inositol trisphosphate receptor: phosphorylation by protein kinase C and calcium calmodulin-dependent protein kinases in reconstituted lipid vesicles, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 2232–2235.
[71] N. Matter, M.F. Ritz, S. Freyermuth, P. Rogue, A.N. Malviya, Stimulation of nuclear protein kinase C leads to phosphorylation of nuclear inositol 1,4,5-trisphosphate receptor and accelerated calcium release by inositol 1,4,5-trisphosphate from isolated rat liver nuclei, J. Biol. Chem. 268 (1993) 732–736.
[72] T. Koga, Y. Yoshida, J.Q. Cai, M.O. Islam, S. Imai, Purification and characterization of 240-kDa cGMP-dependent protein kinase substrate of vascular smooth muscle. Close resemblance to inositol 1,4,5-trisphosphate receptor, J. Biol. Chem. 269 (1994) 11640–11647.
[73] T.A. Rooney, S.K. Joseph, C. Queen, A.P. Thomas, Cyclic GMP induces oscillatory calcium signals in rat hepatocytes, J. Biol. Chem. 271 (1996) 19817–19825.
[74] P. Komalavilas, T.M. Lincoln, Phosphorylation of the inositol 1,4,5-trisphosphate receptor by cyclic GMP-dependent protein kinase, J. Biol. Chem. 269 (1994) 8701–8707.
[75] P. Komalavilas, T.M. Lincoln, 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, J. Biol. Chem. 271 (1996) 21933–21938.
[76] T. Miyakawa, A. Mizushima, K. Hirose, T. Yamazawa, I. Bezprozvanny, T. Kurosaki, M. Iino, Ca^{2+}-sensor region of IP_3 receptor controls intracellular Ca^{2+} signaling, EMBO J. 20 (2001) 1674–1680.
[77] H. Tu, E. Nosyreva, T. Miyakawa, Z. Wang, A. Mizushima, M. Iino, I. Bezprozvanny, Functional and biochemical analysis of the type 1 inositol (1,4,5)-trisphosphate receptor calcium sensor, Biophys. J. 85 (2003) 290–299.
[78] R.L. Patterson, D. Boehning, S.H. Snyder, Inositol 1,4,5-trisphosphate receptors as signal integrators, Ann. Rev. Biochem. 73 (2004) 437–465.
[79] T. Michikawa, H. Hamanaka, H. Otsu, A. Yamamoto, A. Miyawaki, T. Furuichi, Y. Tashiro, K. Mikoshiba, Transmembrane topology and sites of N-glycosylation of inositol 1,4,5-trisphosphate receptor, J. Biol. Chem. 269 (1994) 9184–9189.
[80] G.A. Mignery, T.C. Sudhof, K. Takei, P. De Camilli, Putative receptor for inositol 1,4,5-trisphosphate similar to ryanodine receptor, Nature 342 (1989) 192–195.
[81] D.L. Galvan, E. Borrego-Diaz, P.J. Perez, G.A. Mignery, Subunit oligomerization, and topology of the inositol 1,4,5-trisphosphate receptor, J. Biol. Chem. 274 (1999) 29483–29492.
[82] J. Ramos-Franco, D. Galvan, G.A. Mignery, M. Fill, Location of the permeation pathway in the recombinant type 1 inositol 1,4,5-trisphosphate receptor, J. Gen. Physiol. 114 (1999) 243–250.
[83] P.K. Shah, R. Sowdhamini, Structural understanding of the transmembrane domains of inositol triphosphate receptors and ryanodine receptors towards calcium channeling, Protein Eng. 14 (2001) 867–874.
[84] A.J. Williams, D.J. West, R. Sitsapesan, 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. 34 (2001) 61–104.
[85] D.A. Doyle, J. Morais Cabral, R.A. Pfuetzner, A. Kuo, J.M. Gulbis, S.L. Cohen, B.T. Chait, R. MacKinnon, The structure of the potassium channel: molecular basis of K^+ conduction and selectivity, Science 280 (1998) 69–77.
[86] D. Balshaw, L. Gao, G. Meissner, Luminal loop of the ryanodine receptor: a pore-forming segment, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 3345–3347.
[87] D. Boehning, S.K. Joseph, Functional properties of recombinant type I and type III inositol 1, 4,5-trisphosphate receptor isoforms expressed in COS-7 cells, J. Biol. Chem. 275 (2000) 21492–21499.
[88] D. Boehning, D.O. Mak, J.K. Foskett, S.K. Joseph, Molecular determinants of ion permeation and selectivity in inositol 1,4,5-trisphosphate receptor Ca^{2+} channels, J. Biol. Chem. 276 (2001) 13509–13512.
[89] Y. Jiang, A. Lee, J. Chen, M. Cadene, B.T. Chait, R. MacKinnon, Crystal structure and mechanism of a calcium-gated potassium channel, Nature 417 (2002) 515–522.
[90] Y. Jiang, A. Lee, J. Chen, M. Cadene, B.T. Chait, R. MacKinnon, The open pore conformation of potassium channels, Nature 417 (2002) 523–526.
[91] Y. Jiang, A. Lee, J. Chen, V. Ruta, M. Cadene, B.T. Chait, R. MacKinnon, X-ray structure of a voltage-dependent K^+ channel, Nature 423 (2003) 33–41.
[92] Y. Jiang, V. Ruta, J. Chen, A. Lee, R. MacKinnon, The principle of gating charge movement in a voltage-dependent K^+ channel, Nature 423 (2003) 42–48.
[93] Y.S. Liu, P. Sompornpisut, E. Perozo, Structure of the KcsA channel intracellular gate in the open state, Nat. Struct. Biol. 8 (2001) 883–887.
[94] M. Zhou, J.H. Morais-Cabral, S. Mann, R. MacKinnon, Potassium channel receptor site for the inactivation gate and quaternary amine inhibitors, Nature 411 (2001) 657–661.
[95] A. Kuo, J.M. Gulbis, J.F. Antcliff, T. Rahman, E.D. Lowe, J. Zimmer, J. Cuthbertson, F.M. Ashcroft, T. Ezaki, D.A. Doyle,

Crystal structure of the potassium channel KirBac1.1 in the closed state, Science 300 (2003) 1922–1926.
[96] S.M. Webster, D. Del Camino, J.P. Dekker, G. Yellen, Intracellular gate opening in Shaker K^+ channels defined by high-affinity metal bridges, Nature 428 (2004) 864–868.
[97] S.K. Joseph, S. Pierson, S. Samanta, Trypsin digestion of the inositol trisphosphate receptor: implications for the conformation and domain organization of the protein, Biochem. J. 307 (Pt. 3) (1995) 859–865.
[98] S. Nakade, N. Maeda, K. Mikoshiba, Involvement of the C-terminus of the inositol 1,4,5-trisphosphate receptor in Ca^{2+} release analysed using region-specific monoclonal antibodies, Biochem. J. 277 (Pt. 1) (1991) 125–131.
[99] T. Nakayama, M. Hattori, K. Uchida, T. Nakamura, Y. Tateishi, H. Bannai, M. Iwai, T. Michikawa, T. Inoue, K. Mikoshiba, The regulatory domain of the inositol 1,4,5-trisphosphate receptor is necessary to keep the channel domain closed: possible physiological significance of specific cleavage by caspase 3, Biochem. J. 377 (2004) 299–307.
[100] S.K. Joseph, D. Boehning, S. Pierson, C.V. Nicchitta, Membrane insertion, glycosylation, and oligomerization of inositol trisphosphate receptors in a cell-free translation system, J. Biol. Chem. 272 (1997) 1579–1588.
[101] D.L. Galvan, G.A. Mignery, Carboxyl-terminal sequences critical for inositol 1,4,5-trisphosphate receptor subunit assembly, J. Biol. Chem. 277 (2002) 48248–48260.
[102] M.D. Varnum, W.N. Zagotta, Interdomain interactions underlying activation of cyclic nucleotide-gated channels, Science 278 (1997) 110–113.
[103] S.E. Gordon, M.D. Varnum, W.N. Zagotta, Direct interaction between amino- and carboxyl-terminal domains of cyclic nucleotide-gated channels, Neuron 19 (1997) 431–441.
[104] T. Rosenbaum, S.E. Gordon, Dissecting intersubunit contacts in cyclic nucleotide-gated ion channels, Neuron 33 (2002) 703–713.
[105] C.T. Schulteis, N. Nagaya, D.M. Papazian, Intersubunit interaction between amino- and carboxyl-terminal cysteine residues in tetrameric shaker K^+ channels, Biochemistry 35 (1996) 12133–12140.
[106] S.J. Tucker, F.M. Ashcroft, Mapping of the physical interaction between the intracellular domains of an inwardly rectifying potassium channel, Kir6.2, J. Biol. Chem. 274 (1999) 33393–33397.
[107] N. Maeda, M. Niinobe, K. Mikoshiba, A cerebellar Purkinje cell marker P400 protein is an inositol 1,4,5-trisphosphate ($InsP_3$) receptor protein. Purification and characterization of $InsP_3$ receptor complex, EMBO J. 9 (1990) 61–67.
[108] C.C. Chadwick, A. Saito, S. Fleischer, Isolation and characterization of the inositol trisphosphate receptor from smooth muscle, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 2132–2136.
[109] K. Hamada, T. Miyata, K. Mayanagi, J. Hirota, K. Mikoshiba, Two-state conformational changes in inositol 1,4,5-trisphosphate receptor regulated by calcium, J. Biol. Chem. 277 (2002) 21115–21118.
[110] E. Katayama, H. Funahashi, T. Michikawa, T. Shiraishi, T. Ikemoto, M. Iino, K. Mikoshiba, Native structure and arrangement of inositol-1,4,5-trisphosphate receptor molecules in bovine cerebellar Purkinje cells as studied by quick-freeze deep-etch electron microscopy, EMBO J. 15 (1996) 4844–4851.
[111] K. Takei, G.A. Mignery, E. Mugnaini, T.C. Sudhof, P. De Camilli, Inositol 1,4,5-trisphosphate receptor causes formation of ER cisternal stacks in transfected fibroblasts and in cerebellar Purkinje cells, Neuron 12 (1994) 327–342.
[112] J.S. Marchant, C.W. Taylor, Cooperative activation of IP_3 receptors by sequential binding of IP_3 and Ca^{2+} safeguards against spontaneous activity, Curr. Biol. 7 (1997) 510–518.
[113] C.E. Adkins, C.W. Taylor, Lateral inhibition of inositol 1,4,5-trisphosphate receptors by cytosolic Ca^{2+}, Curr. Biol. 9 (1999) 1115–1118.
[114] N. Nadif Kasri, G. Bultynck, I. Sienaert, G. Callewaert, C. Erneux, L. Missiaen, J.B. Parys, H. De Smedt, The role of calmodulin for inositol 1,4,5-trisphosphate receptor function, Biochim. Biophys. Acta 1600 (2002) 19–31.
[115] K. Mikoshiba, M. Huchet, J.P. Changeux, Biochemical and immunological studies on the P400 protein, a protein characteristic of the Purkinje cell from mouse and rat cerebellum, Dev. Neurosci. 2 (1979) 254–275.