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Intracellular calcium channels: inositol-1,4,5-trisphosphate receptors

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Abstract

The inositol-1,4,5-trisphosphate receptors ($InsP_3Rs$) are the major intracellular Ca^{2+}-release channels in cells. Activity of $InsP_3Rs$ is essential for elementary and global Ca^{2+} events in the cell. There are three $InsP_3Rs$ isoforms that are present in mammalian cells. In this review review we will focus primarily on $InsP_3R$ type 1. The $InsP_3R1$ is a predominant isoform in neurons and it is most extensively studied isoform. Combination of biophysical and structural methods revealed key mechanisms of $InsP_3R$ function and modulation. Cell biological and biochemical studies lead to identification of a large number of $InsP_3R$-binding proteins. $InsP_3Rs$ are involved in the regulation of numerous physiological processes, including learning and memory, proliferation, differentiation, development and cell death. Malfunction of $InsP_3R1$ play a role in a number of neurodegenerative disorders and other disease states. $InsP_3Rs$ represent a potentially valuable drug target for treatment of these disorders and for modulating activity of neurons and other cells. Future studies will provide better understanding of physiological functions of $InsP_3Rs$ in health and disease.

Keywords

Inositol 1,4,5-trisphosphate receptors; cell nucleus; Ca^{2+} signalling; neurodegeneration

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1. Introduction

The inositol-1,4,5-trisphosphate receptors ($InsP_3Rs$) are the major intracellular Ca^{2+}-release channels in cells. The investigation of mechanisms of inositol-1,4,5-trisphosphate ($InsP_3$)-induced Ca^{2+}-release started in 1980s and $InsP_3R$ was first solubilized and purified from the rat cerebellum in 1988 by Snyder's group (Supattapone et al., 1988). Reconstitution of the purified receptor into lipid vesicles showed that $InsP_3$ and other inositol phosphates stimulate calcium flux (Ferris et al., 1989). Then cDNA of $InsP_3R$ was cloned (Furuichi et al., 1989; Mignery et al., 1990) that helps to initiate structure-function studies (Mignery and Sudhof, 1990; Miyawaki et al., 1991). Reconstitution of $InsP_3R$ into planar bilayer membranes revealed its single channel permeability, its modulation by Ca^{2+} and by ATP (Bezprozvanny and Ehrlich, 1993, 1994; Bezprozvanny et al., 1991). Since these initial publications the functional properties of native and recombinant $InsP_3R$ have been extensively characterized by Ca^{2+} flux measurements, planar lipid bilayer or nuclear envelope patch-clamp recordings (Bezprozvanny, 2005; Foskett et al., 2007; Mikoshiba, 2007; Wagner and Yule, 2012). Although some of these studies initially resulted in conflicting data, an agreement has been reached by the field regarding key $InsP_3R$ functional properties.

The variety of $InsP_3$-activated Ca^{2+} channels, including three mammalian $InsP_3R$ isoforms ($InsP_3R$ type 1 ($InsP_3R1$), $InsP_3R$ type 2 ($InsP_3R2$), $InsP_3R$ type 3 ($InsP_3R3$), different slicing variants of IP_3R1, *Drosophila melanogaster* $InsP_3R$ and *Caenorhabditis elegans* $InsP_3R$ have been discovered and characterised (reviewed in (Bezprozvanny, 2005; Foskett et al., 2007; Mikoshiba, 2007)). The three mammalian $InsP_3R$ isoforms are 60–70% identical in sequence (Furuichi et al., 1994) and share a common domain structure (Mignery and Sudhof, 1990; Miyawaki et al., 1991) that consists of an amino-terminal $InsP_3$-binding domain, a carboxyl-terminal Ca^{2+} channel domain, and a middle coupling domain containing most of the putative regulatory sites and is the most divergent (Fig. 1). $InsP_3R1$ is predominant in the central nervous system, but most other tissues express at least two and often all three $InsP_3R$ isoforms at different ratios (Taylor et al., 1999).

The $InsP_3R$ are subjected to multiple levels of regulation (Bezprozvanny, 2005; Foskett et al., 2007; Mikoshiba, 2007; Wagner and Yule, 2012). $InsP_3Rs$ are the targets of a number of allosteric regulators, including protein kinases, adenine nucleotides, pH and divalent cations, all of which may play a part in $InsP_3$-induced Ca^{2+} signaling. Significant effect of phosphorylation on $InsP_3R$ is also well documented (Bezprozvanny, 2005; Foskett et al., 2007; Mikoshiba, 2007). Many protein binding with $InsP_3R$ have been described, and physiological relevance of these interactions is under intense investigation.

At this moment one can find thousands of papers from different research groups dedicated to various aspects of $InsP_3R$ structure, regulation or functional role, but there are still many questions remain to be answered. In this review we focus on $InsP_3R$ type 1, which are predominant isoform expressed in mammalian neurons. Here we will briefly review the structure and basic properties of these channels, their role in the cell functions and in several neurodegenerative disorders, such as Hungtington's disease, spinocerebellar ataxias and Alzheimer's disease.

2. $InsP_3Rs$ in cell functions

A rise in intracellular calcium in neurons in response to $InsP_3Rs$ activation is implicated in the control of a numerous cellular functions, including neurotransmission and synaptic plasticity, proliferation, differentiation, development, gene expression, and cell death (Berridge et al., 1998), Evidence at both cellular and behavioral levels implicates $InsP_3Rs$ in memory formation, in particular they are required during long-term memory (Baker et al., 2013). It was demonstrated that $InsP_3R1$ is extremely important in embryonic development. $InsP_3R1$ knock-out mice have severe ataxia and tonic or tonic-clonic seizures and die by the weaning period (Matsumoto et al., 1996). Besides, $InsP_3R1$ is a critical regulator of synaptic circuit maintenance in the mature cerebellum; this mechanism may underlie motor coordination and learning in adults (Sugawara et al., 2013). Thus, $InsP_3R1$ are essential for proper brain development and function.

$InsP_3R1$ are highly concentrated in the Purkinje cells of the cerebellum, with lower levels being found in other regions of the brain (Sharp et al., 1993a; Sharp et al., 1993b; Taylor et al., 1999) and in a variety of peripheral tissues (Taylor et al., 1999). Immunohistochemical studies in Purkinje cells, *Xenopus* oocytes and pancreatic epithelial cells have revealed that at a subcellular level $InsP_3Rs$ are localized in the rough and smooth endoplasmic reticulum (ER), Golgi complex and nuclear envelope, but not mitochondria or plasma membranes (Lam and Galione, 2013; Ross et al., 1989; Solovyova and Verkhratsky, 2003). Though, it has been indicated that the plasma membrane in some cell types may also contain $InsP_3R$ (Barrera et al., 2004; Dellis et al., 2006; Tanimura et al., 2000), but the role of such localization is rather contradictory.

$InsP_3$ is not the only regulator of $InsP_3Rs$ function; Ca^{2+} plays a critical role in shaping the $InsP_3R$-evoked Ca^{2+} signals. Low Ca^{2+} concentrations (<300 nM) activate the channel and increase its open probability, whereas high Ca^{2+} concentrations inhibit channel opening (Bezprozvanny et al., 1991; Finch et al., 1991; Iino, 1990). These positive and negative feedback cycles are well suited for generating Ca^{2+} oscillations or waves. It appears that $InsP_3Rs$ are activated by simultaneous binding of the two agonists, $InsP_3$ and Ca^{2+}, to the cytoplasmic domain of the molecule that leads to the conformational change in the receptor complex and an increase in the frequency of its Ca^{2+} channel opening, resulting in Ca^{2+} release from the intracellular stores. Both $InsP_3$ and Ca^{2+} are the two main intracellular messengers with their own regulatory pathways (Berridge, 2009, 2012; Decrock et al., 2013). So $InsP_3R$ acts as a skilled “analyst” which coordinates two complex streams of signals and forms an integrated response.

Because of complex Ca^{2+}-mediated feedback on $InsP_3R$ activity, Ca^{2+} signals evoked by the receptor activation are complex, restricted in space and time, and this spatiotemporal organization determines physiological effect of the signal (Berridge, 1997; Bootman et al., 2001; Konieczny et al., 2012). Intracellular $InsP_3$-activated Ca^{2+} signals are organized at three levels, each of them provides different signaling functions and serves as a building block for Ca^{2+} signals at the next level (Berridge, 1996, 1997; Bootman et al., 2001). At the first, so called “fundamental”, level signals result from openings of a single $InsP_3R$ channel. At the resting conditions the cytosolic concentration of Ca^{2+} is low and $InsP_3Rs$ are in a

conformation with low affinity for $InsP_3$, but the activating stimuli trigger the rise in the intracellular Ca^{2+} concentration and the production of $InsP_3$ from the plasma membrane. These low concentrations of the two agonists activate one $InsP_3R$ leading to a rapid localized Ca^{2+}flux called “blip” (Parker and Yao, 1996). At the next, “elementary”, level Ca^{2+} signals, so called “puffs”, arise from the concerted opening of multiple $InsP_3R$ channels. It has been demonstrated that $InsP_3Rs$ are initially randomly distributed in the membranes, but low concentrations of $InsP_3$ cause them to aggregate rapidly and reversibly into clusters (Yao et al., 1995). There is no agreement about the number of $InsP_3Rs$ in a cluster, it was proposed that from four (Rahman, 2012; Rahman and Taylor, 2009) to 25 or 35 $InsP_3Rs$ can open simultaneously during puffs (Shuai et al., 2006; Shuai and Jung, 2003; Solovey and Dawson, 2010). At resting cytosolic Ca^{2+} concentrations clustered $InsP_3Rs$ open independently, but at increasing of Ca^{2+} they are more likely to open and close together (Dickinson et al., 2012; Taylor et al., 2009; Yamasaki-Mann et al., 2013). Ca^{2+}release from one channel acts as an activating ligand to stimulate nearby channels through Ca^{2+}-induced Ca^{2+}release (CICR). So, the spatial organization of $InsP_3R$ channels within clusters and the distribution of clusters, together with the positive regulation by $InsP_3$ and Ca^{2+}, enable local and long-range Ca^{2+}signals to be constructed from the activities of single $InsP_3R$. At intermediate $InsP_3$ concentrations activate groups of $InsP_3Rs$ which release Ca^{2+} to form puffs, but at high concentrations of $InsP_3$ all the receptors are excitable and puffs then act as initiation sites to spawn a regenerative wave that spreads through the cell by CICR (Berridge, 2009). It is the third level of the intracellular Ca^{2+} hierarchy, which is associated with stronger extra-cellular agonist stimulation. Ca^{2+} released at one cluster can trigger Ca^{2+} release at adjacent clusters by CICR, leading to the generation of Ca^{2+} waves that propagate in a saltatory manner in the whole cell.

The nuclear envelope is the Ca^{2+} store with $InsP_3Rs$ in its inner membrane (Fedorenko OA, 2008; Marchenko et al., 2005). It was indicated that the cell nucleus contains the whole set of enzymes required for the $InsR_3$ synthesis from diphosphoinositolphosphate of the nuclear membrane, so the regulation of nuclear $InsP_3Rs$ cannot depend on cytoplasmic processes (Gomes et al., 2006; Klein and Malviya, 2008; Rodrigues et al., 2009). Of course, in small cells $InsR_3$ can freely penetrate into the nucleus by diffusion through the nuclear pores, but in large cells the distance from the plasma membrane to the nucleus is large enough so it is unlikely that $InsP_3Rs$ in the nuclear membrane are activated by cytosolic $InsP_3R$. This point is proved by the fact that that numerous $InsP_3$-activated channels were recorded from the inner nuclear membrane of Purkinje and CA1 pyramidal neurons, which are the largest cells in the brain. No $InsP_3Rs$ were found in the nuclear membrane of granule neurons of the cerebellum (Marchenko et al., 2005) and dentate gyrus (Fedorenko OA, 2007). Therefore the mechanism of regulation of nuclear Ca^{2+} may vary in different cells. There is a growing body of evidence that nuclear Ca^{2+} can affect gene transcription (Bading, 2000; Bengtson and Bading, 2012; Greer and Greenberg, 2008; Parekh and Muallem, 2011; Wiegert and Bading, 2011) through activation of nuclear Ca^{2+}-sensitive kinases and phosphatases, or through direct interaction with Ca^{2+}-dependent transcription factors, such as CREB and DREAM.. However, many issues needed to be clarified, in particular the regulation of Ca^{2+} signals between the cytoplasm and the nucleus and the mechanisms of the intranuclear Ca^{2+} signaling.

3. Biophysical properties of $InsP_3R1$

Biophysical properties of $InsP_3R1$ have been studied following their isolation and reconstitution into artificial lipid planar bilayer membranes (Bezprozvanny and Ehrlich, 1993, 1994; Bezprozvanny et al., 1991; Tu et al., 2005b) and using patch-clamp recordings from nuclei isolated from a variety of cells, including mammalian neurons (Fedorenko OA, 2008; Mak and Foskett, 1997; Marchenko et al., 2005; Wagner and Yule, 2012).

The $InsP_3R$ channel is a Ca^{2+}-selective channel, which is also permeable to other cations such as K^+ and $Ba^{2+}$with permeability ratios P_{Ba}/P_K = 5–6 and P_{Ca}/P_K = 4–5 in symmetrical 140–150 mM K^+ solutions, with relatively little selectivity among different divalent cations (Bezprozvanny and Ehrlich, 1994; Boehning et al., 2001; Marchenko et al., 2005). The $InsP_3R$ are channels with a large single-channel monovalent ion conductance. $InsP_3Rs$ recorded in the inner nuclear membrane of rat cerebellar Purkinje cell have a slope conductance of 355 pS (Marchenko et al., 2005) in symmetric solutions with 150 mM K^+ and the absence of Mg^{2+}. Similar conductance in the same conditions was recorded for expressed recombinant rat $InsP_3R1$ present in COS-7 cell nuclei (Boehning et al., 2001). Single-channel conductance of $InsP_3Rs$ with Ba^{2+} as the current carrier is 121 pS based on nuclear patch measurements (Marchenko et al., 2005). Conductance and selectivity of $InsP_3Rs$ from the nuclear membranes are similar to cerebellar $InsP_3Rs$ and recombinant $InsP_3R1$ incorporated into artificial lipid bilayers (Bezprozvanny and Ehrlich, 1994; Tu et al., 2005b)

At a membrane potential 60 mV in symmetrical 150 mm KCl solution open probability (P_o) for $InsP_3R$ recorded from the nuclear membranes of Purkinje neurons is about 0.036; with Ba^{2+} as a current carrier under the same conditions P_o increases to 0.32 (Marchenko et al., 2005). The presence of Ca^{2+} in solutions in concentrations ≥10 mM strongly decreased the P_o to 0.0023 and that apparently results from the inhibitory effect of high Ca^{2+} concentrations in the vicinity of the receptor produced by Ca^{2+} current through the channel rather than an effect of the presence of Ca^{2+} at the luminal surface of the membrane, because the P_o of single inward K^+ currents remained practically unchanged (Bezprozvanny and Ehrlich, 1994).

$InsP_3Rs$ in the inner nuclear membrane of CA1 pyramidal neurons are activated by IP_3 in concentrations ≥50 nM and saturated (P_o ≥ 95% of P_o at 10 μM IP_3) in concentrations of ≥2 μM in the presence of 250 nm free Ca^{2+} (Fig. 2A, 2B) (Marchenko et al., 2005). $InsP_3$, and other ligands such as ATP, regulate channel activity mainly by modifying the sensitivity of the channels to Ca^{2+} regulation, though in the absence of $InsP_3$, Ca^{2+} alone (20 nm–50 мm) is unable to activate the channels. The regulation of the receptors by Ca^{2+} evokes much controversy (Mak et al., 1998). It has been reported that at steady-state conditions and saturating concentration of $InsP_3$ cerebellar $InsP_3Rs$ incorporated into artificial lipid bilayers were activated by Ca^{2+} at low concentrations and inhibited by higher Ca^{2+} concentration with the peak of activation renged from 200 to 400 nm (Bezprozvanny et al., 1991). In nuclear patch-clamp recordings Ca^{2+} inhibited $InsP_3Rs$ with the same efficiency both at low (0.3 мm) and saturated (10 мm) $InsP_3$ concentrations (Fig. 2C, 2D). Therefore the inhibitory effect of Ca^{2+} on $InsP_3Rs$ does not depend on the $InsP_3$ concentration (Marchenko et al.,

2005). Experiments with flash photolysis of caged $InsP_3$ in Purkinje neurons support these data. It has been shown that Ca^{2+} entry through plasmalemmal Ca^{2+} channels strongly suppressed Ca^{2+} release from stores induced by high (25 мm) $InsP_3$ concentrations (Khodakhah and Ogden, 1995). Recombinant $InsP_3Rs1$ expressed in insect cells also demonstrated a bell-shaped Ca^{2+} dependence at saturated $InsP_3$ concentrations (Tu et al., 2005a). Thus, most recent results support "narrow" Ca^{2+} dependence of $InsP_3R1$ within physiological range of Ca^{2+} concentrations. Besides being activated by $InsP_3$ and suitable concentrations of Ca^{2+}, $InsP_3R$ channel activity is also allosterically potentiated by ATP, (Bezprozvanny and Ehrlich, 1993; Mak et al., 1999; Wagner and Yule, 2012). It has been reported that other nucleotides, such ADP (Iino, 1991), AMP (Ferris et al., 1990), and GTP (Bezprozvanny and Ehrlich, 1993; Mak et al., 1999) can also potentiate $InsP_3R1$ channel activity.

$InsP_3Rs$ have several phosphorylation sites (Bezprozvanny, 2005). $InsP_3Rs$ are regulated by numerous kinases, including cAMP-dependent protein kinase (PKA) (DeSouza et al., 2002; Tang et al., 2003b; Vanderheyden et al., 2009; Wagner et al., 2004; Wagner et al., 2003), protein kinase C (Ferris et al., 1991), cGMP-dependent protein kinase (Haug et al., 1999; Komalavilas and Lincoln, 1994) and others. It has been reported, for example, that phosphorylation by PKA of recombinant type 1 $InsP_3Rs$ expressed in insect cells increased the Po of the channels incorporated into artificial lipid bilayers more than 10-fold (from <2–3% to 30–40%) and increased their sensitivity to InsP3 about 4-fold (Tang et al., 2003b).

There are about 40 proteins or even more that can bind $InsP_3R$ (Fig. 1), among them as calmodulin (Kasri et al., 2004a; Michikawa et al., 1999; Yamada et al., 1995), RACK1 (Woodard et al., 2010), protein 4.1N (Maximov et al., 2003), IRBIT (Mikoshiba, 2012), Bcl-2 (Chen et al., 2004), AKAP9 (Tu et al., 2004) and may others. Therefore, $InsP_3Rs$ in cells act as a critical "signaling hub" that mediate cross-talk between Ca^{2+} signaling, kinases, phosphatases and protein-protein interaction mechanisms. Not surprisingly, abnormality in modulation or activity of $InsP_3R1$ is connected with variety of neurological disorders (see below).

4. Structural studies of $InsP_3Rs$

In the past decade, significant advancements have been made in determining atomic-resolution structures of $InsP_3Rs$. We now have a good understanding of the molecular mechanism underlying receptor recognition of the $InsP_3$ molecule and how this binding is transformed into a protein conformational change at the NH_2-terminus, essential for the initial step of channel activation. The first high-resolution structure determined by X-ray crystallography was the NH_2-terminal $InsP_3$-binding core (IBC) of $InsP_3R1$ (residues 224–604) in complex with $InsP_3$ (Bosanac et al., 2002). The structure of the IBC contains two structurally distinct domains: the β-domain (IBC-β) and α-domain (IBC-α). The IBC-β (residues 224 – 436) adopts a β-trefoil fold comprising 12 β-strands and two single turn helices, whereas the IBC-α (residues 437– 604) adopts an armadillo repeat fold consisting of 8 α-helices (Fig. 3A). The IBC forms an L-shaped structure with the two domains oriented approximately perpendicular to each other; several basic amino acids cluster in a cleft formed by both domains, comprising the $InsP_3$ binding site (Bosanac et al., 2002). The

crystal structures of the NH_2-terminal suppressor domain (SD) have been determined for $InsP_3R1$ (residues 1–223) (Bosanac et al., 2005) and $InsP_3R3$ (residues 1–224) (Chan et al., 2010); moreover, the two structures are nearly identical showing a backbone root mean square deviation (rmsd) of ~1.3 Å (Fig. 3B). The SD folds into a hammer-like structure with a 12 β-stranded "head" domain and a helix-turn-helix "arm" domain. Furthermore, the head domain of the SD adopts a similar β-trefoil fold as found in the IBC. The $InsP_3$ binding affinity of the entire NH_2-terminal region (NT; residues 1– 604 of $InsP_3R1$) is reduced by more than one order of magnitude compared with that of the IBC alone, implying that the SD inhibits or "suppresses" $InsP_3$ binding (Yoshikawa et al., 1996). Evidence suggests that not only is the SD required for suppression of $InsP_3$ binding, but it is also needed for $InsP_3$-induced allosteric channel gating. For example, $InsP_3R1$ lacking the SD shows no measureable $InsP_3$-evoked Ca^{2+} release (Uchida et al., 2003), and remarkably, a single Tyr167Ala mutation in the SD completely abolishes $InsP_3$-evoked Ca^{2+} release (Yamazaki et al., 2010).

Recently, four atomic-resolution NT structures of $InsP_3R1$ have been determined. Lin et al., solved two NT structures of rat $InsP_3R1$ at 3.8 Å resolution; moreover, they derived one structure in an $InsP_3$-free state (*i.e.*, apo) and a second structure in an $InsP_3$-bound state (*i.e.*, holo) from a single crystal grown in the presence of $InsP_3$ (Lin et al., 2011). Subsequently, apo and holo NT structures of rat $InsP_3R1$ at higher resolution were separately determined from crystals grown in the absence (3.0 Å) and presence (3.6 Å) of $InsP_3$, respectively (Seo et al., 2012) (Fig. 3C). The individual structures of the three domains comprising NT of $InsP_3R1$ (*i.e.*, SD, IBC-β, and IBC-α) are highly similar to the separately determined SD (Bosanac et al., 2005) and IBC (Bosanac et al., 2002). Nonetheless, these NT structures represent a significant advancement in the understanding of $InsP_3R$ function by revealing the arrangement of the SD and IBC domains with respect to one another and providing important clues on the bases for tetrameric channel formation. The three domains in NT form a triangular architecture, and the SD is located on the opposite face of the $InsP_3$-binding site, suggesting that the SD suppresses $InsP_3$ binding by an allosteric mechanism. The SD interacts with both the IBC-β and IBC-α, forming two interfaces (*i.e.*, β-interface and α-interface, respectively) (Fig. 3D). The functional importance of residues associated with the α-interface is demonstrated by the Val33Lys mutation, which almost completely abrogates the effects of the SD on $InsP_3$ binding and attenuates the maximal open probability of the full-length channel (Bosanac et al., 2005; Rossi et al., 2009). The most marked conformational change caused by $InsP_3$ binding is the significant decrease in the domain orientation angle between IBC-β and IBC-α (Fig.3C). This ligand binding-induced structural change in IBC occurs with the hinge region between IBC-β and IBC-α set as the pivot point, resulting in a narrowing of the $InsP_3$-binding cleft and a clam-like closure. Consequently, the SD rotates (~9°) towards the IBC, accompanied by a swing that is approximately perpendicular to the IBC 'clam closure' (Fig. 3C).

Recently, Ludtke et al. determined the cryo-EM structure of $InsP_3R$ isolated from the rat cerebellum at 9.5 Å resolution (Ludtke et al., 2011; Murray et al., 2013), dramatically improving our view of the tetrameric full-length $InsP_3R$ channel compared to earlier studies (da Fonseca et al., 2003; Hamada et al., 2002; Jiang et al., 2002; Sato et al., 2004; Serysheva

et al., 2003). The tetrameric structure of $InsP_3R1$ revealed a mushroom-shaped overall architecture with a fourfold symmetry axis along a central plug (Fig. 3D). The cytoplasmic region is located apical to the transmembrane domain with several large openings or "windows" between the two regions (Fig. 3E) and consists of a relatively rigid exterior surface with a more structurally variable and probably flexible interior (*i.e.* exhibiting higher statistical variability in the calculated density map), possibly due to multiple conformations of $InsP_3R1$ in the closed state. Docking the high resolution crystal structures of $InsP_3R1$-NT into this full-length cryo-EM structure shows that $InsP_3R1$-NT forms a tetrameric ring around the fourfold symmetry axis, with the hot spot loop (HS-loop; so-called due to the high structural homology with a loop where a cluster of disease-associated mutations in ryanodine receptor domain A have been mapped) of $InsP_3R1$ (residues 165–180) involved in intersubunit interactions (Fig. 3E). Importantly, Li et al. produced covalently linked tetrameric $InsP_3R1$-NT through site-specific cysteine insertions in the intersubunit interface that was modeled in the docking studies and demonstrated that $InsP_3$ inhibits cross-linked tetrameric $InsP_3R1$-NT in a concentration-dependent manner, suggesting that $InsP_3$ binding closes the clam-like IBC, disrupting these intersubunit interactions and allowing the channel to open (Fig. 4C) (Li et al., 2013). Taken together, these observations indicate that the $InsP_3R1$-NT plays a critical role in the allosteric modulation of ion channel conductance through a modification of quaternary arrangement.

Very recently, the structure of $InsP_3R1$-NT in complex with calcium binding protein 1 (CaBP1) was determined using nuclear magnetic resonance (NMR) spectroscopy-based chemical shift perturbation mapping and paramagnetic relaxation enhancement (PRE) data (Fig. 4A) (Li et al., 2013). The structure showed that exposed hydrophobic residues in Ca^{2+}-bound CaBP1 (*i.e.* Val101, Leu104, and Val162) interact with clustered hydrophobic residues (*i.e.* Leu302, Ile364, and Leu393) in the IBC-β domain of $InsP_3R1$ (Fig. 4A). Superimposing the structure of the $InsP_3R1$-NT/CaBP1 complex with the tetrameric $InsP_3R1$-NT shown in Fig. 4B revealed that the four molecules of CaBP1 form a ring-like structure around the central cytosolic vestibule. Further, chemical cross-linking experiments showed that CaBP1 enhances the production of cross-linked tetrameric $InsP_3R1$-NT (Li et al., 2013). In aggregate with data demonstrating that Ca^{2+}-CaBP1 inhibits $InsP_3$-evoked Ca^{2+} release (Haynes et al., 2004; Kasri et al., 2004b; Li et al., 2013) a novel regulatory molecular mechanism has been proposed where CaBP1 clamps the intersubunit interactions and thereby inhibits channel opening (Fig. 4C) (Li et al., 2013).

5. The role of $InsP_3Rs$ in neurodegenerative diseases

The role of $InsP_3R1$ has been shown for several neurodegenerative diseases such as Huntington's disease, spinocerebellar ataxias (SCAs) and Alzheimer disease (AD) (Bezprozvanny, 2009, 2011; Bezprozvanny and Mattson, 2008; Kasumu and Bezprozvanny, 2012).

HD is an autosomal-dominant neurodegenerative disorder and it is caused by polyglutamine (polyQ) expansion in the amino-terminal region of a protein huntingtin (Htt). Huntington disease (HD) is an autosomal-dominant neurodegenerative disorder with the age of onset between 35 and 50 years and inexorable progression to death 15–20 years after onset. The

symptoms include motor abnormalities including chorea and psychiatric disturbance with gradual dementia (Vonsattel and DiFiglia, 1998). Neuropathological analysis reveals selective and progressive neuronal loss in the striatum (caudate nucleus, putamen and globus pallidus) (Vonsattel and DiFiglia, 1998; Vonsattel et al., 1985). GABAergic medium spiny striatal neurons (MSN) are the most sensitive to neuronal degeneration in HD (Vonsattel and DiFiglia, 1998; Vonsattel et al., 1985). It is widely accepted that mutant Htt (mHtt) protein acquires a “toxic gain of function” (Tobin and Signer, 2000). A a number of potential “toxic functions” of mutant Htt have been suggested. Our laboratory is focused on connection between mutant Htt and deregulated neuronal Ca^{2+} signaling. We first discovered that mHtt binds directly and specifically to the C-terminal region of the type 1 IP_3 receptor (IP_3R1) (Tang et al., 2003a). Recently, unbiased high-throughput screening assays confirmed mHtt binding to IP_3R1 (Kaltenbach et al., 2007). Interestingly the affinity of mHtt to IP_3R1 increases when mHtt is associated with HAP1A (Tang et al., 2003a). Moreover, mHtt, but not normal Htt, augmented IP_3R1 activity in planar lipid bilayers (Tang et al., 2003a). Similarly, application of subthreshold concentrations of DHPG, an mGluR1/5 agonist, sensitized Ca^{2+} release in YAC128 primary MSN cultures (Tang et al., 2003a). This is consistent with the fact that glutamate-induced apoptosis of MSNs from YAC128 mice is mediated by mGluR1/5 and NR2B receptors (Tang et al., 2005). In fact, specific blockade of IP_3R1 with 2-APB and Enoxaparin is neuroprotective in the same model (Tang et al., 2005). In more recent experiments, we demonstrated that viral delivery of a peptide that disrupts mHtt association with IP_3R1 protected YAC128 MSNs *in vitro* and *in vivo* (Tang et al., 2009). We also reported that Ca^{2+} stabilizing agent dantrolene exerts beneficial effects in mouse model of HD (Chen et al., 2011). We also demonstrated that continuous overactivation of $InsP_3R1$ pathway causes compensatory upregulation of neuronal store-operated Ca^{2+} entry in HD neurons, which contributes to Ca^{2+} overload and pathology (Wu et al., 2011). The alterations in ER enzymes that have been observed in HD postmortem brains (Kerner et al., 1997; Mao and Wang, 2001, 2002; Tallaksen-Greene et al., 1998; Testa et al., 1995) are consistent with malfunction of ER Ca^{2+} handling in HD MSN neurons. In recent studies it was discovered that $InsP_3R1$ association of ER stress chaperone protein GRP78 is impaired in HD R6/2 model mice, resulting in misregulation of $InsP_3R1$ gating (Higo et al., 2010). These results provide additional support for the role of $InsP_3R1$ in HD pathology.

Many SCAs are caused by polyQ-expansion in ataxin proteins and typically affect cerebellar Purkinje cells. SCAs are generally characterized by cerebellar atrophy and a progressive incoordination of movement known as ataxia (Filla et al., 1999; Lastres-Becker et al., 2008; Schols et al., 2004). Although there is a phenotypic overlap with cerebellar atrophy and ataxia in all SCA patients, other brain regions may be affected in different type of disease. The pathogenesis of SCAs is not fully understood, however, several different pathogenic mechanisms have been studied in SCAs such as dysregulation of transcription and gene expression, alterations in calcium homeostasis and synaptic neurotransmission, mitochondrial stress and apoptosis (reviewed in (Bezprozvanny and Klockgether, 2010; Carlson et al., 2009; Kasumu and Bezprozvanny, 2012; Matilla-Duenas et al., 2009)).

Studies in our laboratory primarily focused on the role of $InsP_3Rs$ in pathogenesis of SCA type 2 (SCA2). The SCA2 is caused by an expansion and translation of unstable glutamine (Q) repeats in the gene encoding ataxin-2 from the normal 22 to more than 31 extra glutamine repeats (Imbert et al., 1996; Pulst et al., 1996; Sanpei et al., 1996). PolyQ-expanded ataxin-2 (ATXN2exp) protein, similar to wildtype ataxin-2 (ATXNwt), is widely expressed in the body tissues. Similar to wildtype ataxin-2, polyglutamine-expanded ataxin-2 protein is ubiquitously expressed in cells without severe aggregation and formation of inclusion bodies (Huynh et al., 2000). Cerebellar PCs in SCA2 patients are mostly affected with a loss of over 75% (Schols et al., 2004). The role of calcium signaling in the pathogenesis of SCA2 is supported by the genetic association between polymorphisms in the CACNA1A gene and the age of disease onset in patients diagnosed with SCA2 (Pulst et al., 2005). The role of aberrant neuronal calcium signaling in SCA2 pathogenesis was strengthened further by a finding in our lab that $ATX2^{exp}$ but not wildtype ATX2 ($ATX2^{wt}$) specifically binds $InsP_3R1$ (Liu et al., 2009). In a lipid bilayer reconstitution experiment, we found the presence of $ATX2^{exp}$ substantially sensitized IP_3R 1 to activation by $InsP_3$ (Liu et al., 2009). Consistent with involvement of excitotoxic mechanism, we discovered that PC cells in SCA2 mouse undergo dark cell degeneration (DCD) mode of cell death (Kasumu and Bezprozvanny, 2012). Long-term feeding of SCA2-58Q mice with a calcium stabilizer dantrolene alleviated the age-dependent motor coordination deficits and PC cell loss in these mice (Liu et al., 2009). More recently we performed partial suppression of $InsP_3$-induced Ca^{2+} release in PC cells of SCA2-58Q mice by using inositol 5-phosphatase viral construct (Kasumu et al., 2012). We discovered that this approach prevented the onset of PC dysfunction, alleviated motor incoordination, and reduced age-dependent PC degeneration in SCA2-58Q mice (Kasumu et al., 2012). These results indicate that partial suppression of $InsP_3$-induced Ca^{2+} release is a viable therapeutic strategy for treatment of SCA2 and possibly other SCAs (Kasumu et al., 2012). Remarkably, insufficient $InsP_3R$-mediated Ca^{2+} signaling also leads to the ataxic phenotype and PC degeneration, such as observed in SCA15/16 patients haploinsufficient for the $InsP_3R1$ gene (Hara et al., 2008; Iwaki et al., 2008; van de Leemput et al., 2007). An ataxic phenotype is also observed in *opt* mice with reduced levels of $InsP_3R1$ protein (Street et al., 1997) and a severe ataxia is observed in $InsP_3R1$ knockout mice (Matsumoto et al., 1996). These findings indicate that the reduced Ca^{2+} release via $InsP_3R1$ also leads to PC dysfunction and ataxic phenotype and that there is a relatively narrow range of optimal $InsP_3$-mediated Ca^{2+} signaling that is compatible with proper function and long-term survival of PCs. Deviation from this optimal range in either direction of $InsP_3$-mediated Ca^{2+} signaling results in PC dysfunction and an ataxic phenotype (Kasumu et al., 2012).

Alzheimer disease (AD) is another neurodegenerative disease in whose pathogenesis abnormal $InsP_3R$ signaling is likely to be involved. AD progresses slowly and affects neurons mainly in the cortex and hippocampus. The exact mechanism of AD pathogenesis is not known. One to two percent of AD cases constitute early onset familial Alzheimer disease cases. FAD is caused by mutations in genes coding presenilin 1 (PS1), presenilin 2 (PS2) or amyloid precursor proteins (APP). The first observation of exaggerated $InsP_3R$-mediated Ca^{2+} release from ER has been detected in fibroblasts from AD patients (Ito et al., 1994). Later similar results have been obtained in AD mouse models as well as in *Xenopus*

oocytes expressing human PS1 and PS2 mutant constructs (Bezprozvanny and Mattson, 2008; Stutzmann, 2007). The mechanism of abnormal $InsP_3R$ signaling in AD is not fully understood. Our laboratory discovered that presenilns function as ER Ca^{2+} leak channels, and FAD mutations in presenilins result in elevated ER Ca^{2+} levels (Nelson et al., 2007; Tu et al., 2006; Zhang et al., 2010). Increased ER Ca^{2+} levels are in turn result in enhanced $InsP_3R1$-mediated Ca^{2+} release. The role of presenilins as ER Ca^{2+} leak channels was recently confirmed in unbiased screen for modulators of calcium homeostasis (Bandara et al., 2013). An alternative hypothesis is that mutant presenilins enhance $InsP_3R1$ gating via direct protein-protein interaction (Cheung et al., 2010; Cheung et al., 2008). Future studies will be need to understand the mechanism of $InsP_3R1$ disregulation in AD and its importance for pathology.

6. Conclusions

A remarkable progress has been made since original discovery of $InsP_3R$ family 25 years ago. Using electrophysiological and molecular methods we dissected major functional properties of all 3 mammalian $InsP_3R$ isoforms. Thanks to application of structural biology methods we start to understand function of $InsP_3R$ as a “molecular machine” in atomic details. Identification of a large number of $InsP_3R$-binding partners highlighted a role of $InsP_3R$ as a key “signaling hub” in cells. We discovered that dysfunction of $InsP_3R$ is linked to a variety of neurodegenerative disorders, and demonstarted that $InsP_3R$ is a potential target for therapeutic interference in these and other disorders. There is no doubt that further research of $InsP_3R$ family in healthy and disease states will continue to provide additional valuable insights into basic biology and medicine.

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References

Bading H. Transcription-dependent neuronal plasticity the nuclear calcium hypothesis. Eur J Biochem. 2000; 267:5280–5283. [PubMed: 10951185]

Baker KD, Edwards TM, Rickard NS. The role of intracellular calcium stores in synaptic plasticity and memory consolidation. Neurosci Biobehav Rev. 2013; 37:1211–1239. [PubMed: 23639769]

Bandara S, Malmersjo S, Meyer T. Regulators of Calcium Homeostasis Identified by Inference of Kinetic Model Parameters from Live Single Cells Perturbed by siRNA. Science signaling. 2013; 6:ra56. [PubMed: 23838183]

Barrera NP, Morales B, Villalon M. Plasma and intracellular membrane inositol 1,4,5-trisphosphate receptors mediate the Ca(2+) increase associated with the ATP-induced increase in ciliary beat frequency. Am J Physiol Cell Physiol. 2004; 287:C1114–C1124. [PubMed: 15175223]

Bengtson CP, Bading H. Nuclear calcium signaling. Adv Exp Med Biol. 2012; 970:377–405. [PubMed: 22351065]

Berridge MJ. Microdomains and elemental events in calcium signalling. Cell Calcium. 1996; 20:95–96. [PubMed: 8889200]

Berridge MJ. Elementary and global aspects of calcium signalling. J Exp Biol. 1997; 200:315–319. [PubMed: 9050239]

Berridge MJ. Inositol trisphosphate and calcium signalling mechanisms. Biochim Biophys Acta. 2009; 1793:933–940. [PubMed: 19010359]

Berridge MJ. Calcium signalling remodelling and disease. Biochem Soc Trans. 2012; 40:297–309. [PubMed: 22435804]

Berridge MJ, Bootman MD, Lipp P. Calcium - a life and death signal. Nature. 1998; 395:645–648. [PubMed: 9790183]

Bezprozvanny I. The inositol 1,4,5-trisphosphate receptors. Cell Calcium. 2005; 38:261–272. [PubMed: 16102823]

Bezprozvanny I. Calcium signaling and neurodegenerative diseases. Trends Mol Med. 2009; 15:89–100. [PubMed: 19230774]

Bezprozvanny I. Role of inositol 1,4,5-trisphosphate receptors in pathogenesis of Huntington's disease and spinocerebellar ataxias. Neurochem Res. 2011; 36:1186–1197. [PubMed: 21210219]

Bezprozvanny I, Ehrlich BE. ATP modulates the function of inositol 1,4,5-trisphosphategated channels at two sites. Neuron. 1993; 10:1175–1184. [PubMed: 7686381]

Bezprozvanny I, Ehrlich BE. Inositol (1,4,5)-trisphosphate (InsP3)-gated Ca channels from cerebellum: conduction properties for divalent cations and regulation by intraluminal calcium. J. Gen. Physiol. 1994; 104:821–856. [PubMed: 7876825]

Bezprozvanny I, Klockgether T. Therapeutic prospects for spinocerebellar ataxia type 2 and 3. Drugs of the Future. 2010; 34:991–999.

Bezprozvanny I, Mattson MP. Neuronal calcium mishandling and the pathogenesis of Alzheimer's disease. Trends Neurosci. 2008; 31:454–463. [PubMed: 18675468]

Bezprozvanny I, Watras J, Ehrlich BE. Bell-shaped calcium-response curves of Ins(1,4,5)P_3- and calcium-gated channels from endoplasmic reticulum of cerebellum. Nature. 1991; 351:751–754. [PubMed: 1648178]

Boehning D, Joseph SK, Mak DO, Foskett JK. Single-channel recordings of recombinant inositol trisphosphate receptors in mammalian nuclear envelope. Biophys J. 2001; 81:117–124. [PubMed: 11423400]

Bootman MD, Lipp P, Berridge MJ. The organisation and functions of local Ca(2+) signals. J Cell Sci. 2001; 114:2213–2222. [PubMed: 11493661]

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]

Bosanac I, Yamazaki H, Matsu-Ura T, Michikawa T, Mikoshiba K, Ikura M. Crystal structure of the ligand binding suppressor domain of type 1 inositol 1,4,5-trisphosphate receptor. Molecular cell. 2005; 17:193–203. [PubMed: 15664189]

Carlson KM, Andresen JM, Orr HT. Emerging pathogenic pathways in the spinocerebellar ataxias. Curr Opin Genet Dev. 2009; 19:247–253. [PubMed: 19345087]

Chan J, Yamazaki H, Ishiyama N, Seo MD, Mal TK, Michikawa T, Mikoshiba K, Ikura M. Structural studies of inositol 1,4,5-trisphosphate receptor: coupling ligand binding to channel gating. J Biol Chem. 2010; 285:36092–36099. [PubMed: 20843799]

Chen R, Valencia I, Zhong F, McColl KS, Roderick HL, Bootman MD, Berridge MJ, Conway SJ, Holmes AB, Mignery GA, Velez P, Distelhorst CW. Bcl-2 functionally interacts with inositol 1,4,5-trisphosphate receptors to regulate calcium release from the ER in response to inositol 1,4,5-trisphosphate. J Cell Biol. 2004; 166:193–203. [PubMed: 15263017]

Chen X, Wu J, Lvovskaya S, Herndon E, Supnet C, Bezprozvanny I. Dantrolene is neuroprotective in Huntington's disease transgenic mouse model. Mol Neurodegener. 2011; 6:81. [PubMed: 22118545]

Cheung KH, Mei L, Mak DO, Hayashi I, Iwatsubo T, Kang DE, Foskett JK. Gain-of-function enhancement of IP3 receptor modal gating by familial Alzheimer's disease-linked presenilin mutants in human cells and mouse neurons. Sci Signal. 2010; 3:ra22. [PubMed: 20332427]

Cheung KH, Shineman D, Muller M, Cardenas C, Mei L, Yang J, Tomita T, Iwatsubo T, Lee VM, Foskett JK. Mechanism of Ca2+ disruption in Alzheimer's disease by presenilin regulation of InsP(3) receptor channel gating. Neuron. 2008; 58:871–883. [PubMed: 18579078]

da Fonseca PC, Morris SA, Nerou EP, Taylor CW, Morris EP. 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. 2003; 100:3936–3941. [PubMed: 12651956]

Decrock E, De Bock M, Wang N, Gadicherla AK, Bol M, Delvaeye T, Vandenabeele P, Vinken M, Bultynck G, Krysko DV, Leybaert L. IP3, a small molecule with a powerful message. Biochim Biophys Acta. 2013; 1833:1772–1786. [PubMed: 23291251]

Dellis O, Dedos SG, Tovey SC, Taufiq Ur R, Dubel SJ, Taylor CW. Ca2+ entry through plasma membrane IP3 receptors. Science. 2006; 313:229–233. [PubMed: 16840702]

DeSouza N, Reiken S, Ondrias K, Yang YM, Matkovich S, Marks AR. Protein Kinase A and Two Phosphatases Are Components of the Inositol 1,4,5-Trisphosphate Receptor Macromolecular Signaling Complex. J Biol Chem. 2002; 277:39397–39400. [PubMed: 12167631]

Dickinson GD, Swaminathan D, Parker I. The probability of triggering calcium puffs is linearly related to the number of inositol trisphosphate receptors in a cluster. Biophys J. 2012; 102:1826–1836. [PubMed: 22768938]

Fedorenko OA DD, Marchenko SM. Nuclear ionic channels of the granule cells from the dentate gyrus. Fiziol Zh. 2007; 53:9–15. [PubMed: 17725038]

Fedorenko OA DD, Marchenko SM. Calcium channels in the nuclear envelope of pyramidal neurons of the hippocampus. Neurophysiology. 2008; 40:238–242.

Ferris CD, Huganir RL, Bredt DS, Cameron AM, Snyder SH. Inositol trisphosphate receptor - phosphorylation by protein kinase-C and calcium calmodulin-dependent protein kinases in reconstituted lipid vesicles. Proc. Natl. Acad. Sci. USA. 1991; 88:2232–2235. [PubMed: 1848697]

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. USA. 1990; 87:2147–2151. [PubMed: 2156262]

Ferris CD, Huganir RL, Supattapone S, Snyder SH. Purified inositol 1,4,5- trisphosphate receptor mediates calcium flux in reconstituted lipid vesicles. Nature. 1989; 342:87–89. [PubMed: 2554143]

Filla A, De Michele G, Santoro L, Calabrese O, Castaldo I, Giuffrida S, Restivo D, Serlenga L, Condorelli DF, Bonuccelli U, Scala R, Coppola G, Caruso G, Cocozza S. Spinocerebellar ataxia type 2 in southern Italy: a clinical and molecular study of 30 families. J Neurol. 1999; 246:467–471. [PubMed: 10431773]

Finch EA, Turner TJ, Goldin SM. Calcium as a coagonist of inositol 1,4,5-trisphosphate-induced calcium release. Science. 1991; 252:443–446. [PubMed: 2017683]

Foskett JK, White C, Cheung KH, Mak DO. Inositol Trisphosphate Receptor Ca2+ Release Channels. Physiological reviews. 2007; 87:593–658. [PubMed: 17429043]

Furuichi T, Kohda K, Miyawaki A, Mikoshiba K. Intracellular channels. Current Opinion Neurobiol. 1994; 4:294–303.

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; 342:32–38. [PubMed: 2554142]

Gomes DA, Leite MF, Bennett AM, Nathanson MH. Calcium signaling in the nucleus. Can J Physiol Pharmacol. 2006; 84:325–332. [PubMed: 16902580]

Greer PL, Greenberg ME. From synapse to nucleus: calcium-dependent gene transcription in the control of synapse development and function. Neuron. 2008; 59:846–860. [PubMed: 18817726]

Hamada K, Miyata T, Mayanagi K, Hirota J, Mikoshiba K. Two-state conformational changes in inositol 1,4,5-trisphosphate receptor regulated by calcium. J Biol Chem. 2002; 277:21115–21118. [PubMed: 11980893]

Hara K, Shiga A, Nozaki H, Mitsui J, Takahashi Y, Ishiguro H, Yomono H, Kurisaki H, Goto J, Ikeuchi T, Tsuji S, Nishizawa M, Onodera O. Total deletion and a missense mutation of ITPR1 in Japanese SCA15 families. Neurology. 2008; 71:547–551. [PubMed: 18579805]

Haug LS, Jensen V, Hvalby O, Walaas SI, Ostvold AC. Phosphorylation of the inositol 1,4,5-trisphosphate receptor by cyclic nucleotide-dependent kinases in vitro and in rat cerebellar slices in situ. J Biol Chem. 1999; 274:7467–7473. [PubMed: 10066812]

Haynes LP, Tepikin AV, Burgoyne RD. Calcium-binding protein 1 is an inhibitor of agonist-evoked, inositol 1,4,5-trisphosphate-mediated calcium signaling. J Biol Chem. 2004; 279:547–555. [PubMed: 14570872]

Higo T, Hamada K, Hisatsune C, Nukina N, Hashikawa T, Hattori M, Nakamura T, Mikoshiba K. Mechanism of ER stress-induced brain damage by IP(3) receptor. Neuron. 2010; 68:865–878. [PubMed: 21145001]

Huynh DP, Figueroa K, Hoang N, Pulst SM. Nuclear localization or inclusion body formation of ataxin-2 are not necessary for SCA2 pathogenesis in mouse or human. Nat Genet. 2000; 26:44–50. [PubMed: 10973246]

Iino M. Biphasic Ca^{2+} dependence of inositol 1,4,5-trisphosphate-induced Ca release in smooth muscle cells of the guinea pig *taenia caeci*. J. Gen. Physiol. 1990; 95:1103–1122. [PubMed: 2373998]

Iino M. Effects of adenine nucleotides on inositol 1,4,5-trisphosphate-induced calcium release in vascular smooth muscle cells. J. Gen.Physiol. 1991; 98:681–698. [PubMed: 1660060]

Imbert G, Saudou F, Yvert G, Devys D, Trottier Y, Garnier JM, Weber C, Mandel JL, Cancel G, Abbas N, Durr A, Didierjean O, Stevanin G, Agid Y, Brice A. Cloning of the gene for spinocerebellar ataxia 2 reveals a locus with high sensitivity to expanded CAG/glutamine repeats. Nat Genet. 1996; 14:285–291. [PubMed: 8896557]

Ito E, Oka K, Etcheberrigaray R, Nelson TJ, McPhie DL, Tofel-Grehl B, Gibson GE, Alkon DL. Internal Ca2+ mobilization is altered in fibroblasts from patients with Alzheimer disease. Proc Natl Acad Sci U S A. 1994; 91:534–538. [PubMed: 8290560]

Iwaki A, Kawano Y, Miura S, Shibata H, Matsuse D, Li W, Furuya H, Ohyagi Y, Taniwaki T, Kira J, Fukumaki Y. Heterozygous deletion of ITPR1, but not SUMF1, in spinocerebellar ataxia type 16. J Med Genet. 2008; 45:32–35. [PubMed: 17932120]

Jiang QX, Thrower EC, Chester DW, Ehrlich BE, Sigworth FJ. Three-dimensional structure of the type 1 inositol 1,4,5-trisphosphate receptor at 24 A resolution. The EMBO journal. 2002; 21:3575–3581. [PubMed: 12110570]

Kaltenbach LS, Romero E, Becklin RR, Chettier R, Bell R, Phansalkar A, Strand A, Torcassi C, Savage J, Hurlburt A, Cha GH, Ukani L, Chepanoske CL, Zhen Y, Sahasrabudhe S, Olson J, Kurschner C, Ellerby LM, Peltier JM, Botas J, Hughes RE. Huntingtin interacting proteins are genetic modifiers of neurodegeneration. PLoS Genet. 2007; 3:e82. [PubMed: 17500595]

Kasri NN, Bultynck G, Smyth J, Szlufcik K, Parys JB, Callewaert G, Missiaen L, Fissore RA, Mikoshiba K, de Smedt H. The N-terminal Ca2+-independent calmodulin-binding site on the inositol 1,4,5-trisphosphate receptor is responsible for calmodulin inhibition, even though this inhibition requires Ca2+ Mol Pharmacol. 2004a; 66:276–284. [PubMed: 15266018]

Kasri NN, Holmes AM, Bultynck G, Parys JB, Bootman MD, Rietdorf K, Missiaen L, McDonald F, De Smedt H, Conway SJ, Holmes AB, Berridge MJ, Roderick HL. Regulation of InsP3 receptor activity by neuronal Ca2+-binding proteins. EMBO J. 2004b; 23:312–321. [PubMed: 14685260]

Kasumu A, Bezprozvanny I. Deranged Calcium Signaling in Purkinje Cells and Pathogenesis in Spinocerebellar Ataxia 2 (SCA2) and Other Ataxias. Cerebellum (London, England). 2012; 11:630–639.

Kasumu AW, Liang X, Egorova P, Vorontsova D, Bezprozvanny I. Chronic suppression of inositol 1,4,5-triphosphate receptor-mediated calcium signaling in cerebellar purkinje cells alleviates pathological phenotype in spinocerebellar ataxia 2 mice. J Neurosci. 2012; 32:12786–12796. [PubMed: 22973002]

Kerner JA, Standaert DG, Penney JB Jr, Young AB, Landwehrmeyer GB. Expression of group one metabotropic glutamate receptor subunit mRNAs in neurochemically identified neurons in the rat neostriatum, neocortex, and hippocampus. Brain Res Mol Brain Res. 1997; 48:259–269. [PubMed: 9332723]

Khodakhah K, Ogden D. Fast activation and inactivation of inositol trisphosphate-evoked Ca2+ release in rat cerebellar Purkinje neurones. J Physiol (Lond). 1995; 487:343–358. [PubMed: 8558468]

Klein C, Malviya AN. Mechanism of nuclear calcium signaling by inositol 1,4,5-trisphosphate produced in the nucleus, nuclear located protein kinase C and cyclic AMP-dependent protein kinase. Front Biosci. 2008; 13:1206–1226. [PubMed: 17981624]

Komalavilas P, Lincoln TM. Phosphorylation of the inositol 1,4,5-trisphosphate receptor by cyclic GMP-dependent protein kinase. J Biol Chem. 1994; 269:8701–8707. [PubMed: 8132598]

Konieczny V, Keebler MV, Taylor CW. Spatial organization of intracellular Ca2+ signals. Semin Cell Dev Biol. 2012; 23:172–180. [PubMed: 21925615]

Lam AK, Galione A. The endoplasmic reticulum and junctional membrane communication during calcium signaling. Biochim Biophys Acta. 2013; 1833:2542–2559. [PubMed: 23770047]

Lastres-Becker I, Rub U, Auburger G. Spinocerebellar ataxia 2 (SCA2). Cerebellum. 2008; 7:115–124. [PubMed: 18418684]

Li C, Enomoto M, Rossi AM, Seo MD, Rahman T, Stathopulos PB, Taylor CW, Ikura M, Ames JB. CaBP1, a neuronal Ca2+ sensor protein, inhibits inositol trisphosphate receptors by clamping intersubunit interactions. Proc Natl Acad Sci U S A. 2013; 110:8507–8512. [PubMed: 23650371]

Lin CC, Baek K, Lu Z. Apo and InsP(3)-bound crystal structures of the ligand-binding domain of an InsP(3) receptor. Nat Struct Mol Biol. 2011; 18:1172–1174. [PubMed: 21892169]

Liu J, Tang TS, Tu H, Nelson O, Herndon E, Huynh DP, Pulst SM, Bezprozvanny I. Deranged calcium signaling and neurodegeneration in spinocerebellar ataxia type 2. J Neurosci. 2009; 29:9148–9162. [PubMed: 19625506]

Ludtke SJ, Tran TP, Ngo QT, Moiseenkova-Bell VY, Chiu W, Serysheva II. Flexible architecture of IP3R1 by Cryo-EM. Structure. 2011; 19:1192–1199. [PubMed: 21827954]

Mak DO, Foskett JK. Single-channel kinetics, inactivation, and spatial distribution of inositol trisphosphate (IP3) receptors in Xenopus oocyte nucleus. J Gen Physiol. 1997; 109:571–587. [PubMed: 9154905]

Mak DO, McBride S, Foskett JK. Inositol 1,4,5-trisphosphate activation of inositol trisphosphate receptor Ca2+ channel by ligand tuning of Ca2+ inhibition. Proc. Natl. Acad. Sci. U S A. 1998; 95:15821–15825. [PubMed: 9861054]

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. J Biol Chem. 1999; 274:22231–22237. [PubMed: 10428789]

Mao L, Wang JQ. Upregulation of preprodynorphin and preproenkephalin mRNA expression by selective activation of group I metabotropic glutamate receptors in characterized primary cultures of rat striatal neurons. Brain Res Mol Brain Res. 2001; 86:125–137. [PubMed: 11165379]

Mao L, Wang JQ. Glutamate cascade to cAMP response element-binding protein phosphorylation in cultured striatal neurons through calcium-coupled group I metabotropic glutamate receptors. Mol Pharmacol. 2002; 62:473–484. [PubMed: 12181423]

Marchenko SM, Yarotskyy VV, Kovalenko TN, Kostyuk PG, Thomas RC. Spontaniously active and InsP3-activated ion channels in cell nuclei from rat cerebellar Purkinje and granule neurons. J Physiol. 2005; 563:897–910. [PubMed: 15774532]

Matilla-Duenas A, Sanchez I, Corral-Juan M, Davalos A, Alvarez R, Latorre P. Cellular and Molecular Pathways Triggering Neurodegeneration in the Spinocerebellar Ataxias. Cerebellum. 2009

Matsumoto M, Nakagawa T, Inoue T, Nagata E, Tanaka K, Takano H, Minowa O, Kuno J, Sakakibara S, Yamada M, Yoneshima H, Miyawaki A, Fukuuchi Y, Furuichi T, Okano H, Mikoshiba K, Noda T. Ataxia and epileptic seizures in mice lacking type 1 inositol 1,4,5-trisphosphate receptor. Nature. 1996; 379:168–171. [PubMed: 8538767]

Maximov A, Tang T-S, Bezprozvanny I. Association of the type 1 inositol (1,4,5)-trisphosphate receptor with 4.1N protein in neurons. Molecular Cellular Neuroscience. 2003; 22:271–283. [PubMed: 12676536]

Michikawa T, Hirota J, Kawano S, Hiraoka M, Yamada M, Furuichi T, Mikoshiba K. Calmodulin mediates calcium-dependent inactivation of the cerebellar type 1 inositol 1,4,5-trisphosphate receptor. Neuron. 1999; 23:799–808. [PubMed: 10482245]

Mignery GA, Newton CL, Archer BT, Sudhof TC. Structure and expression of the rat inositol 1,4,5-trisphosphate receptor. J. Biol. Chem. 1990; 265:12679–12685. [PubMed: 2165071]

Mignery GA, Sudhof TC. The ligand binding site and transduction mechanism in the inositol-1,4,5-triphosphate receptor. EMBO J. 1990; 9:3893–3898. [PubMed: 2174351]

Mikoshiba K. IP3 receptor/Ca2+ channel: from discovery to new signaling concepts. J Neurochem. 2007; 102:1426–1446. [PubMed: 17697045]

Mikoshiba K. The discovery and structural investigation of the IP(3) receptor and the associated IRBIT protein. Adv Exp Med Biol. 2012; 740:281–304. [PubMed: 22453947]

Miyawaki A, Furuichi T, Ryou Y, Yoshikawa S, Nakagawa T, Saitoh T, Mikoshiba K. Structure-function relationships of the mouse inositol 1,4,5-trisphosphate receptor. Proc Natl Acad Sci U S A. 1991; 88:4911–4915. [PubMed: 1647021]

Murray SC, Flanagan J, Popova OB, Chiu W, Ludtke SJ, Serysheva II. Validation of cryo-EM structure of IP(3)R1 channel. Structure. 2013; 21:900–909. [PubMed: 23707684]

Nelson O, Tu H, Lei T, Bentahir M, de Strooper B, Bezprozvanny I. Familial Alzheimer disease-linked mutations specifically disrupt Ca2+ leak function of presenilin 1. J Clin Invest. 2007; 117:1230–1239. [PubMed: 17431506]

Parekh AB, Muallem S. Ca(2+) signalling and gene regulation. Cell Calcium. 2011; 49:279. [PubMed: 21429578]

Parker I, Yao Y. Ca2+ transients associated with openings of inositol trisphosphate-gated channels in Xenopus oocytes. J Physiol (Lond). 1996; 491:663–668. [PubMed: 8815201]

Pulst SM, Nechiporuk A, Nechiporuk T, Gispert S, Chen XN, Lopes-Cendes I, Pearlman S, Starkman S, Orozco-Diaz G, Lunkes A, DeJong P, Rouleau GA, Auburger G, Korenberg JR, Figueroa C, Sahba S. Moderate expansion of a normally biallelic trinucleotide repeat in spinocerebellar ataxia type 2. Nat Genet. 1996; 14:269–276. [PubMed: 8896555]

Pulst SM, Santos N, Wang D, Yang H, Huynh D, Velazquez L, Figueroa KP. Spinocerebellar ataxia type 2: polyQ repeat variation in the CACNA1A calcium channel modifies age of onset. Brain. 2005; 128:2297–2303. [PubMed: 16000334]

Rahman T. Dynamic clustering of IP3 receptors by IP3. Biochem Soc Trans. 2012; 40:325–330. [PubMed: 22435806]

Rahman T, Taylor CW. Dynamic regulation of IP3 receptor clustering and activity by IP3. Channels (Austin). 2009; 3:226–232. [PubMed: 19617706]

Rodrigues MA, Gomes DA, Nathanson MH, Leite MF. Nuclear calcium signaling: a cell within a cell. Braz J Med Biol Res. 2009; 42:17–20. [PubMed: 18982194]

Ross CA, Meldolesi J, Milner TA, Satoh T, Supattapone S, Snyder SH. Inositol 1,4,5-trisphosphate receptor localized to endoplasmic reticulum in cerebellar Purkinje neurons. Nature. 1989; 339:468–470. [PubMed: 2542801]

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]

Sanpei K, Takano H, Igarashi S, Sato T, Oyake M, Sasaki H, Wakisaka A, Tashiro K, Ishida Y, Ikeuchi T, Koide R, Saito M, Sato A, Tanaka T, Hanyu S, Takiyama Y, Nishizawa M, Shimizu N, Nomura Y, Segawa M, Iwabuchi K, Eguchi I, Tanaka H, Takahashi H, Tsuji S. Identification of the spinocerebellar ataxia type 2 gene using a direct identification of repeat expansion and cloning technique, DIRECT. Nat Genet. 1996; 14:277–284. [PubMed: 8896556]

Sato C, Hamada K, Ogura T, Miyazawa A, Iwasaki K, Hiroaki Y, Tani K, Terauchi A, Fujiyoshi Y, Mikoshiba K. Inositol 1,4,5-trisphosphate receptor contains multiple cavities and L-shaped ligand-binding domains. J Mol Biol. 2004; 336:155–164. [PubMed: 14741211]

Schols L, Bauer P, Schmidt T, Schulte T, Riess O. Autosomal dominant cerebellar ataxias: clinical features, genetics, and pathogenesis. Lancet Neurol. 2004; 3:291–304. [PubMed: 15099544]

Seo MD, Velamakanni S, Ishiyama N, Stathopulos PB, Rossi AM, Khan SA, Dale P, Li C, Ames JB, Ikura M, Taylor CW. Structural and functional conservation of key domains in InsP3 and ryanodine receptors. Nature. 2012; 483:108–112. [PubMed: 22286060]

Serysheva II, Bare DJ, Ludtke SJ, Kettlun CS, Chiu W, Mignery GA. Structure of the type 1 inositol 1,4,5-trisphosphate receptor revealed by electron cryomicroscopy. J Biol Chem. 2003; 278:21319–21322. [PubMed: 12714606]

Sharp AH, Dawson TM, Ross CA, Fotuhi M, Mourey RJ, Snyder SH. Inositol 1,4,5-trisphosphate receptors: immunohistochemical localization to discrete areas of rat central nervous system. Neuroscience. 1993a; 53:927–942. [PubMed: 8389431]

Sharp AH, McPherson PS, Dawson TM, Aoki C, Campbell KP, Snyder SH. Differential immunohistochemical localization of inositol 1,4,5-trisphosphate- and ryanodinesensitive Ca2+ release channels in rat brain. J Neurosci. 1993b; 13:3051–3063. [PubMed: 8392539]

Shuai J, Rose HJ, Parker I. The number and spatial distribution of IP3 receptors underlying calcium puffs in Xenopus oocytes. Biophys J. 2006; 91:4033–4044. [PubMed: 16980372]

Shuai JW, Jung P. Optimal ion channel clustering for intracellular calcium signaling. Proc Natl Acad Sci U S A. 2003; 100:506–510. [PubMed: 12518049]

Solovey G, Dawson SP. Intra-cluster percolation of calcium signals. PLoS One. 2010; 5:e8997. [PubMed: 20174630]

Solovyova N, Verkhratsky A. Neuronal endoplasmic reticulum acts as a single functional Ca2+ store shared by ryanodine and inositol-1,4,5-trisphosphate receptors as revealed by intra-ER [Ca2+] recordings in single rat sensory neurones. Pflugers Arch. 2003; 446:447–454. [PubMed: 12764616]

Stathopulos PB, Seo MD, Enomoto M, Amador FJ, Ishiyama N, Ikura M. Themes and variations in ER/SR calcium release channels: structure and function. Physiology (Bethesda). 2012; 27:331–342. [PubMed: 23223627]

Street VA, Bosma MM, Demas VP, Regan MR, Lin DD, Robinson LC, Agnew WS, Tempel BL. The type 1 inositol 1,4,5-trisphosphate receptor gene is altered in the opisthotonos mouse. J Neurosci. 1997; 17:635–645. [PubMed: 8987786]

Stutzmann GE. The pathogenesis of Alzheimers disease is it a lifelong "calciumopathy"? Neuroscientist. 2007; 13:546–559. [PubMed: 17901262]

Sugawara T, Hisatsune C, Le TD, Hashikawa T, Hirono M, Hattori M, Nagao S, Mikoshiba K. Type 1 inositol trisphosphate receptor regulates cerebellar circuits by maintaining the spine morphology of purkinje cells in adult mice. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2013; 33:12186–12196. [PubMed: 23884927]

Supattapone S, Worley PF, Baraban JM, Snyder SH. Solubilization, purification, and characterization of an inositol trisphosphate receptor. J. Biol. Chem. 1988; 263:1530–1534. [PubMed: 2826483]

Tallaksen-Greene SJ, Kaatz KW, Romano C, Albin RL. Localization of mGluR1a-like immunoreactivity and mGluR5-like immunoreactivity in identified populations of striatal neurons. Brain Res. 1998; 780:210–217. [PubMed: 9507137]

Tang TS, Guo C, Wang H, Chen X, Bezprozvanny I. Neuroprotective effects of inositol 1,4,5-trisphosphate receptor C-terminal fragment in a Huntington's disease mouse model. J Neurosci. 2009; 29:1257–1266. [PubMed: 19193873]

Tang TS, Slow E, Lupu V, Stavrovskaya IG, Sugimori M, Llinas R, Kristal BS, Hayden MR, Bezprozvanny I. Disturbed Ca2+ signaling and apoptosis of medium spiny neurons in Huntington's disease. Proc Natl Acad Sci U S A. 2005; 102:2602–2607. [PubMed: 15695335]

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. 2003a; 39:227–239. [PubMed: 12873381]

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. 2003b; 23:403–415. [PubMed: 12533600]

Tanimura A, Tojyo Y, Turner RJ. Evidence that type I, II, and III inositol 1,4,5- trisphosphate receptors can occur as integral plasma membrane proteins. J Biol Chem. 2000; 275:27488–27493. [PubMed: 10874040]

Taylor CW, Genazzani AA, Morris SA. Expression of inositol trisphosphate receptors. Cell Calcium. 1999; 26:237–251. [PubMed: 10668562]

Taylor CW, Taufiq Ur R, Pantazaka E. Targeting and clustering of IP3 receptors: key determinants of spatially organized Ca2+ signals. Chaos. 2009; 19:037102. [PubMed: 19798811]

Testa CM, Standaert DG, Landwehrmeyer GB, Penney JB Jr, Young AB. Differential expression of mGluR5 metabotropic glutamate receptor mRNA by rat striatal neurons. J Comp Neurol. 1995; 354:241–252. [PubMed: 7782501]

Tobin AJ, Signer ER. Huntington's disease: the challenge for cell biologists. Trends Cell Biol. 2000; 10:531–536. [PubMed: 11121745]

Tu H, Nelson O, Bezprozvanny A, Wang Z, Lee S-F, Hao YH, Serneels L, De Strooper B, Yu G, Bezprozvanny I. Presenilins form ER calcium leak channels, a function disrupted by mutations linked to familial Alzheimer's disease. Cell. 2006; 126:981–993. [PubMed: 16959576]

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. J Biol Chem. 2004; 279:19375–19382. [PubMed: 14982933]

Tu H, Wang Z, Bezprozvanny I. Modulation of mammalian inositol 1,4,5-trisphosphate receptor isoforms by calcium: a role of calcium sensor region. Biophys J. 2005a; 88:1056–1069. [PubMed: 15531634]

Tu H, Wang Z, Nosyreva E, De Smedt H, Bezprozvanny I. Functional characterization of mammalian inositol 1,4,5-trisphosphate receptor isoforms. Biophys J. 2005b; 88:1046–1055. [PubMed: 15533917]

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]

van de Leemput J, Chandran J, Knight MA, Holtzclaw LA, Scholz S, Cookson MR, Houlden H, Gwinn-Hardy K, Fung HC, Lin X, Hernandez D, Simon-Sanchez J, Wood NW, Giunti P, Rafferty I, Hardy J, Storey E, Gardner RJ, Forrest SM, Fisher EM, Russell JT, Cai H, Singleton AB. Deletion at ITPR1 underlies ataxia in mice and spinocerebellar ataxia 15 in humans. PLoS Genet. 2007; 3:e108. [PubMed: 17590087]

Vanderheyden V, Devogelaere B, Missiaen L, De Smedt H, Bultynck G, Parys JB. Regulation of inositol 1,4,5-trisphosphate-induced Ca2+ release by reversible phosphorylation and dephosphorylation. Biochim Biophys Acta. 2009; 1793:959–970. [PubMed: 19133301]

Vonsattel JP, DiFiglia M. Huntington disease. Journal of neuropathology and experimental neurology. 1998; 57:369–384. [PubMed: 9596408]

Vonsattel JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson EP Jr. Neuropathological classification of Huntington's disease. Journal of neuropathology and experimental neurology. 1985; 44:559–577. [PubMed: 2932539]

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. J Biol Chem. 2004; 279:46242–46252. [PubMed: 15308649]

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. J Biol Chem. 2003; 278:45811–45817. [PubMed: 12939273]

Wagner LE 2nd, Yule DI. Differential regulation of the InsP(3) receptor type-1 and -2 single channel properties by InsP(3), Ca(2)(+) and ATP. J Physiol. 2012; 590:3245–3259. [PubMed: 22547632]

Wiegert JS, Bading H. Activity-dependent calcium signaling and ERK-MAP kinases in neurons: a link to structural plasticity of the nucleus and gene transcription regulation. Cell Calcium. 2011; 49:296–305. [PubMed: 21163523]

Woodard GE, Lopez JJ, Jardin I, Salido GM, Rosado JA. TRPC3 regulates agoniststimulated Ca2+ mobilization by mediating the interaction between type I inositol 1,4,5-trisphosphate receptor, RACK1, and Orai1. J Biol Chem. 2010; 285:8045–8053. [PubMed: 20022948]

Wu J, Shih HP, Vigont V, Hrdlicka L, Diggins L, Singh C, Mahoney M, Chesworth R, Shapiro G, Zimina O, Chen X, Wu Q, Glushankova L, Ahlijanian M, Koenig G, Mozhayeva GN, Kaznacheyeva E, Bezprozvanny I. Neuronal store-operated calcium entry pathway as a novel

therapeutic target for Huntington's disease treatment. Chemistry & biology. 2011; 18:777–793. [PubMed: 21700213]

Yamada M, Miyawaki A, Saito K, Nakajima T, Yamamoto-Hino M, Ryo Y, Furuichi T, Mikoshiba K. The calmodulin-binding domain in the mouse type 1 inositol 1,4,5-trisphosphate receptor. Biochem J. 1995; 308:83–88. [PubMed: 7755592]

Yamasaki-Mann M, Demuro A, Parker I. Cytosolic [Ca2+] regulation of InsP3-evoked puffs. Biochem J. 2013; 449:167–173. [PubMed: 23035871]

Yamazaki H, Chan J, Ikura M, Michikawa T, Mikoshiba K. Tyr-167/Trp-168 in type 1/3 inositol 1,4,5-trisphosphate receptor mediates functional coupling between ligand binding and channel opening. J Biol Chem. 2010; 285:36081–36091. [PubMed: 20813840]

Yao Y, Choi J, Parker I. Quantal puffs of intracellular Ca2+ evoked by inositol trisphosphate in Xenopus oocytes. J Physiol (Lond). 1995; 482:533–553. [PubMed: 7738847]

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. J Biol Chem. 1996; 271:18277–18284. [PubMed: 8663526]

Zhang H, Sun S, Herreman A, De Strooper B, Bezprozvanny I. Role of presenilins in neuronal calcium homeostasis. J Neurosci. 2010; 30:8566–8580. [PubMed: 20573903]

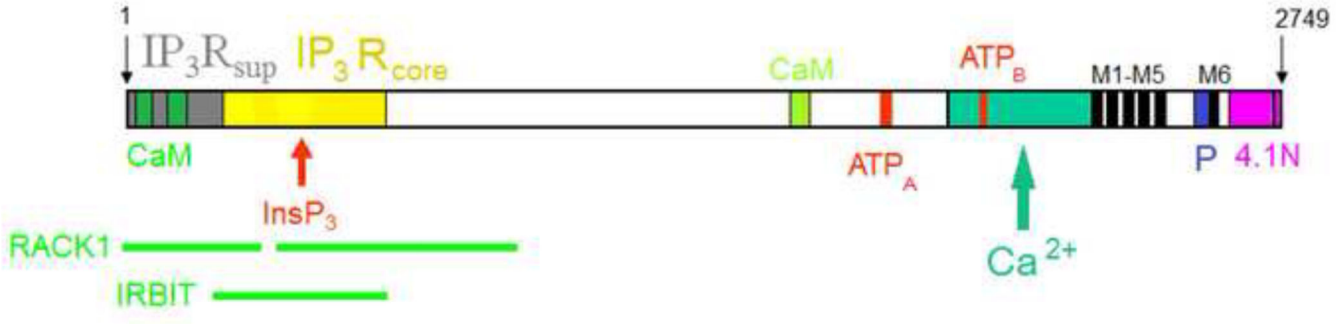

Figure 1. Domain structure of $InsP_3R1$

$InsP_3R_{sup}$ and $InsP_3R_{core}$ domains, CaM, RACK1, IRBIT and 4.1N binding sites, two ATP (A and B) binding sites, the Ca^{2+} sensor region, the M1–M6 transmembrane domains and the pore-forming region (P) are shown.

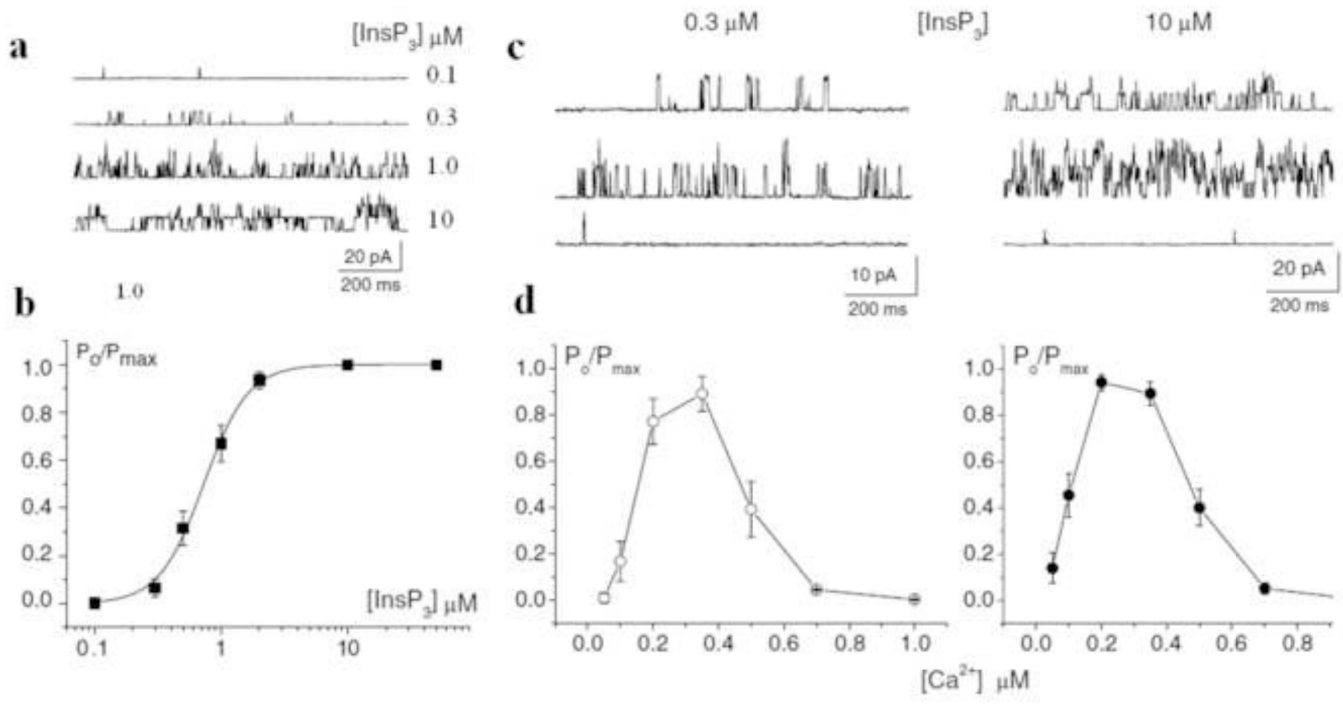

Figure 2. Modulation of $InsP_3R1$ by Ca^{2+} and $InsP_3$

$InsP_3R1$ activity was recorded by patching cerebellar Purkinje cell nuclear membrane (Marchenko et al., 2005)

a, $InsP_3R$ channel activity at different $InsP_3$ concentrations. **b**, dependence of the normalised open probability of $InsP_3R$ channels on $InsP_3$ concentration. Data points represent mean ±SEM of five experiments. The solid curve is the Hill equation fit with EC_{50}=0.68 μM and Hill coefficient =2.5. The channel activity (**c**) and normalised open probability of $InsP_3R$ channels (**d**) at different Ca^{2+} concentrations in the presence of low (0.3 μM, left; n=5) and saturated (10 μM, right; n=7) $InsP_3$ concentrations. At both $InsP_3$ concentrations 1 μM of Ca^{2+} almost completely inhibited the channel activity. Patch pipettes were filled with $BaCl_2$ solution, bath contained standard KCl solution with $[Ca^{2+}]_i$ =250 nM. Holding potential was 40 mV.

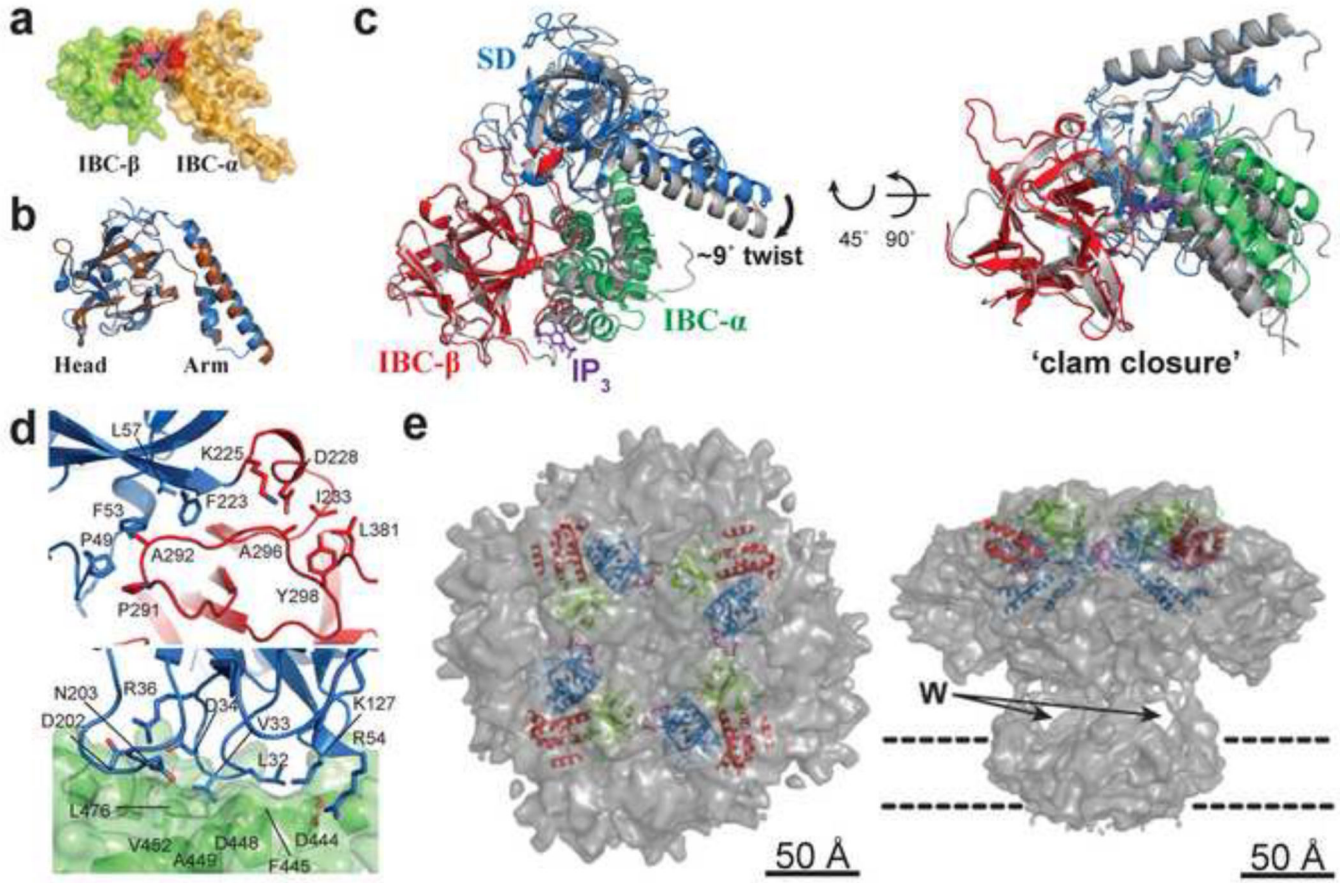

Figure 3. Structure of $InsP_3R1$-NT relative to the full-length tetrameric receptor

a, Crystal structure of the $InsP_3R1$-IBC in complex with $InsP_3$ at 2.2 Å resolution. Ribbon and surface representations of the IBC-β (light green) and IBC-α (orange) are shown. $InsP_3$-coordinating regions are colored red. **b**, Superimposed structures of the $InsP_3R1$-SD (blue) and $InsP_3R3$-SD (brown). **c**, Superimposed apo (SD, blue; IBC-β, red; IBC-α, green) and $InsP_3$-bound (*i.e.* holo) $InsP_3R1$-NT (grey) structures. The structures were aligned by overlaying the IBC-β. **d**, The two interfaces between the SD and IBC (colored as in **c**). The short β-interface (top) consists predominantly of hydrophobic interactions between Pro49, Phe53, and Phe223 from the SD, and Pro291 and Ala292 from the IBC-β, and is supported by a salt bridge between Lys225 and Asp228. The longer α-interface (bottom) is stabilized by hydrophobic interactions between Val33 in the SD and a pocket formed by Val452, Phe445, Ala449, and Leu476 within IBC-α. Electrostatic interactions between Arg54 and Lys127 from the SD and Asp444 from IBC-α are also involved in forming the α-interface. **e**, Cryo-EM structures of full-length $InsP_3R1$ in the closed state are shown from top (left) and side (right) views. The docked crystal structure of $InsP_3R1$-NT is shown as a ribbon representation with SD in blue, IBC-β in red and IBC-α in green. The HS-loop is colored magenta. The windows (W) of $InsP_3R1$ are indicated with arrows. The membrane bilayer boundaries are depicted with broken lines. The figures in **a** and **b** are reproduced from (Stathopulos et al., 2012) and in **c–e** from (Seo et al., 2012).

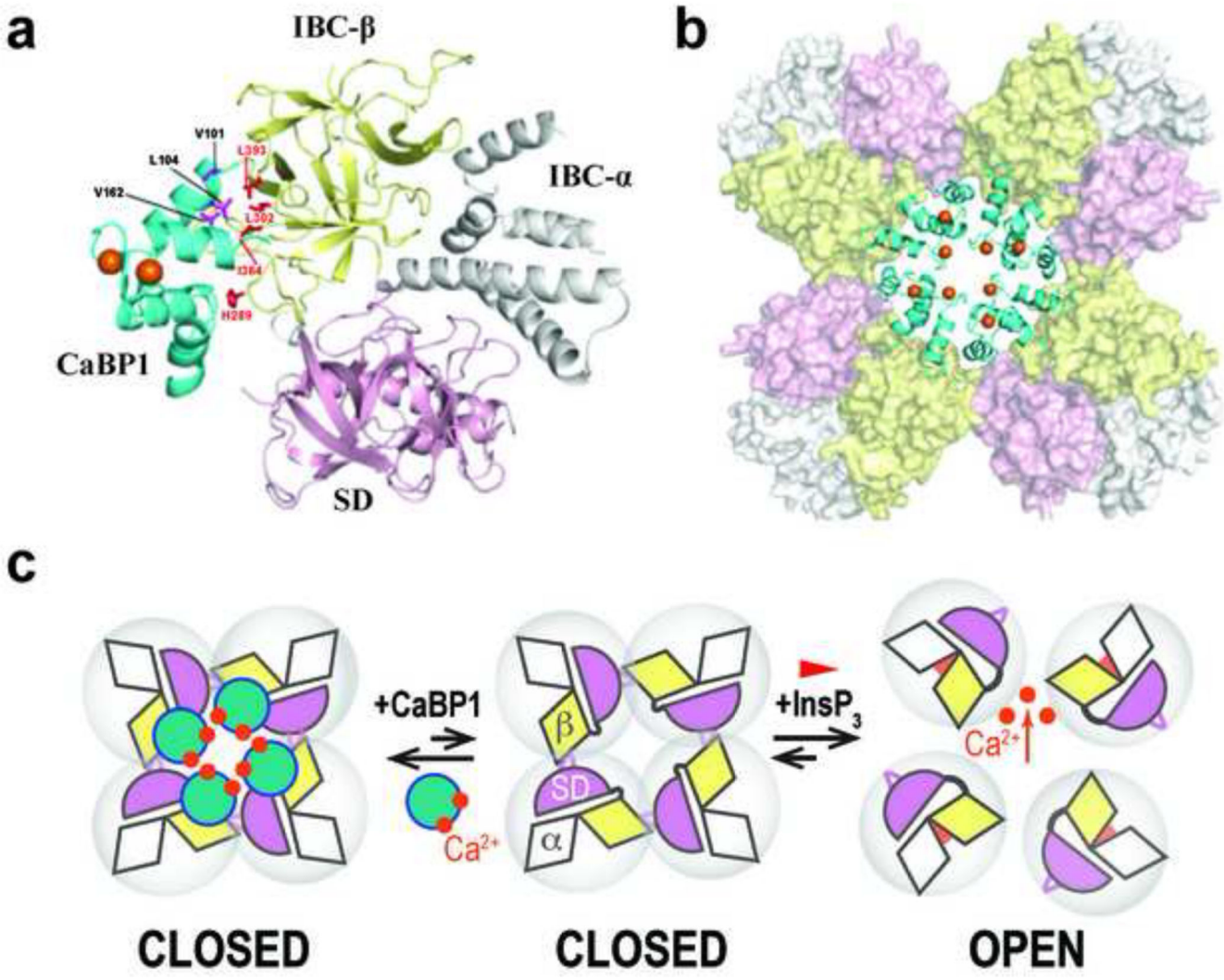

Figure 4. Structure of the $InsP_3R1$-NT/CaBP1 complex and opposing effects of $InsP_3$ and CaBP1 on intersubunit interactions

a, Structure of the C-lobe of CaBP1 (CaBP1-C) bound to $InsP_3R1$-NT in a 1:1 complex. NMR structural restraints were used to define contacts between $InsP_3R1$-NT (SD, pink; IBC-β, yellow; IBC-α, grey) and the CaBP1-C (cyan, with Ca^{2+} atoms colored orange). Key residues at the binding interface are highlighted in magenta (CaBP1) and red ($InsP_3R1$). **b**, Model for the tetrameric $InsP_3R1$-NT/CaBP1-C complex (colored as in **f**) generated by superimposing the $InsP_3R1$-NT crystal structure (Seo et al., 2012) onto the cryo-EM electron density map of $InsP_3R1$ (Ludtke et al., 2011). **c**, Interactions between adjacent $InsP_3R1$-NTs that are mediated by IBC-β (yellow) and the HS-loop of the SD (magenta) hold the tetrameric $InsP_3R1$ in a closed state. $InsP_3$ binding closes the clam-like IBC, disrupting these intersubunit interactions, and allowing the channel to open. CaBP1 clamps the intersubunit interactions associated with the closed-state, thereby inhibiting channel opening. The figures are reproduced from (Li et al., 2013).