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The inositol 1,4,5-trisphosphate receptors

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Abstract

The inositol (1,4,5)-trisphosphate receptors ($InsP_3R$) are the intracellular calcium (Ca^{2+}) release channels that play a key role in Ca^{2+} signaling in cells. Three $InsP_3R$ isoforms—$InsP_3R$ type 1 ($InsP_3R1$), $InsP_3R$ type 2 ($InsP_3R2$), and $InsP_3R$ type 3 ($InsP_3R3$) are expressed in mammals. A single $InsP_3R$ isoform is expressed in *Drosophila melanogaster* ($DmInsP_3R$) and *Caenorhabditis elegans* ($CeInsP_3R$). The progress made during last decade towards understanding the function and the properties of the $InsP_3R$ is briefly reviewed in this chapter. The main emphasis is on studies that revealed structural determinants responsible for the ligand recognition by the $InsP_3R$, ion permeability of the $InsP_3R$, modulation of the $InsP_3R$ by cytosolic Ca^{2+}, ATP and PKA phosphorylation and on the recently identified $InsP_3R$-binding partners. The main focus is on the $InsP_3R1$, but the recent information about properties of other $InsP_3R$ isoforms is also discussed.

Keywords: Calcium signaling; Structure-function; Ion channels; Calcium imaging; Planar lipid bilayers; Mutagenesis

1. Introduction

Ten years ago together with Barbara Ehrlich we published a topical review in the Journal of Membrane Biology [1] in which we summarized major functional properties of the inositol 1,4,5-trisphosphate receptors ($InsP_3R$). In approximately the same time, Teiichi Furuichi, Katsuhiko Mikoshiba and colleagues published a review in Current Opinion in Neurobiology [2], in which they summarized known information about molecular structure of the $InsP_3R$. The main effort during last decade has been focused on trying to merge the "functional" [1] and "molecular" [2] views of the $InsP_3R$ into one coherent image. Here, I will briefly review the progress made so far. From the beginning I would like to apologize to many colleagues whose papers I was not able to discuss here due to space limitations of this review format. A number of laboratories around the world used a variety of experimental systems to perform structure-functional analysis of the $InsP_3R$. The most fruitful approaches turned out to be (1) to analyze Ca^{2+} signals supported by wild-type and mutant $InsP_3R$ expressed in DT40 cell line with all three $InsP_3R$ genes genetically knocked out [3]; (2) to analyze single channel behavior of wild-type and mutant $InsP_3R$ expressed in mammalian cell lines followed by purification and reconstitution into planar lipid bilayers; (3) to analyze single channel behavior of wild-type and mutant $InsP_3R$ expressed in Sf9 cells by baculoviral infection followed by reconstitution into planar lipid bilayers; (4) to analyze single channel behavior of wild-type and mutant $InsP_3R$ expressed in *Xenopus* oocytes by cRNA injection followed by nuclear patch recordings. Due to different approaches used by multiple groups some of the obtained results are controversial, but in this review I will attempt to focus on consensus that has recently began to emerge. The main structure-function effort has been focused on type 1 mammalian $InsP_3R$ ($InsP_3R1$) and I will primarily discuss $InsP_3R1$ results. More recently some information about properties of mammalian $InsP_3R2$, mammalian $InsP_3R3$, *Drosophila melanogaster* $InsP_3R$, and *Caenorhabditis elegans* $InsP_3R$ started to emerge and these data will also be discussed briefly.

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2. $InsP_3R1$

What have we learned about $InsP_3R1$ during the last decade? Here is what we knew in 1995: (1) $InsP_3R1$ channel is a tetramer of four subunits 2749 amino acids (for rat) each; (2) each $InsP_3R1$ subunit composed of amino-terminal $InsP_3$-binding domain, carboxy-terminal channel-forming domain, and the middle coupling (modulatory) domain; (3) $InsP_3R1$ are high conductance moderately divalent cation-selective channels gated by $InsP_3$; (4) $InsP_3R1$ display bell-shaped dependence on cytosolic Ca^{2+} in physiological range of Ca^{2+} concentrations (pCa 8–5); (5) $InsP_3R1$ allosterically potentiated by millimolar ATP; (6) $InsP_3R1$ efficiently phosphorylated by PKA leading to $InsP_3R1$ activation. All of these statements still true in 2005. The main difference is that now we have much more detailed and precise information about structural determinants responsible for the $InsP_3R1$ functional properties, as outlined in this section. Based on limited trypsin proteolysis cerebellar $InsP_3R1$ can be divided into five compact domains (I–V) separated by linker regions [4] (Fig. 1). Whenever possible, I will discuss properties of each of these five domains separately.

Domain I (aa 1–345) contains $InsP_3$ binding supressor domain ($InsP_3R_{sup}$, aa 1–223) (Fig. 1). The structure of $InsP_3R_{sup}$ domain has been recently solved at atomic resolution [5] (Fig. 1). Analysis of obtained results indicated that $InsP_3R_{sup}$ structure corresponds to the β-trefoil type fold [5]. Within its sequence $InsP_3R_{sup}$ domain contains proline-rich motif ^{48}NPPKKFRD^{55} that binds to EVH1 domain in adaptor protein Homer [6] (Fig. 1). Association of $InsP_3R1$ with Homer is important for coupling between $InsP_3R1$ and mGluR1 [6] and for conformational coupling between $InsP_3R1$ and TRPC plasma membrane Ca^{2+} entry channels [7]. In addition to Homer-binding site, $InsP_3R_{sup}$ domain also contains two binding sites for calmodulin (CaM) (aa 49–81 and 106–128) [8–10], a binding site for CaBP1 Ca^{2+}-binding protein (aa 49–81) [11,12] and the first part of the binding site for the RACK1 PKC adaptor protein (aa 1–346) [13] (Fig. 1). Association of CaBP1 protein with $InsP_3R1$ was claimed to activate $InsP_3R1$ in the $InsP_3$-independent manner [11], but other laboratories reported that CaBP1 protein inhibits $InsP_3R1$ [12,14]. The physiological relevance of $InsP_3R_{sup}$ association with CaM and RACK1 is under investigation.

Domain II (aa 346–923) contains the bulk of the $InsP_3$ core binding domain ($InsP_3R_{core}$, aa 224–604) [15]. When expressed in isolation, $InsP_3R_{core}$ domain forms high affinity (k_d = 2.3 nM) $InsP_3$ binding site [15]. In the presence of $InsP_3R_{sup}$ domain the binding affinity of $InsP_3R_{core}$ domain is attenuated to a physiological range (k_d = 45 nM) [16]. The structure of $InsP_3R_{core}$ domain complexed with $InsP_3$ has been recently determined [17] (Fig. 1). The $InsP_3R_{core}$ structure consists of amino-terminal (aa 224–436) "β-trefoil-type"

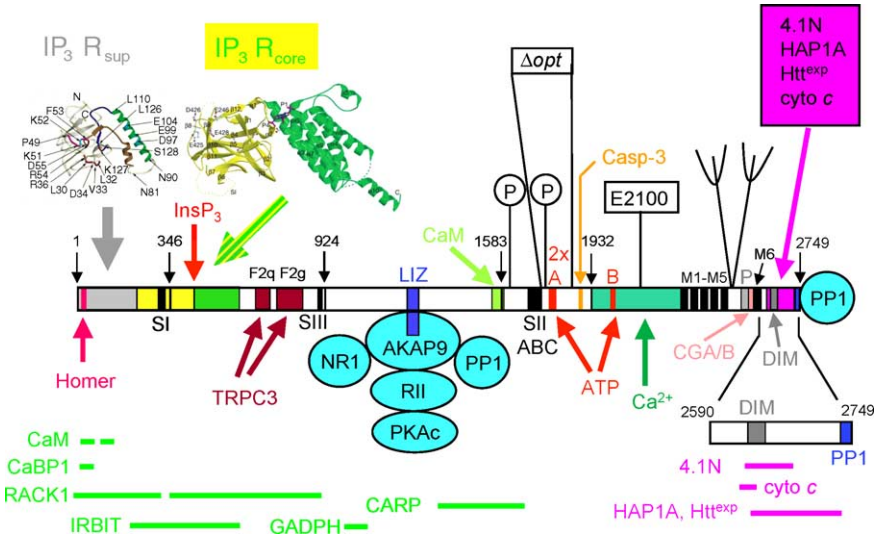

Fig. 1. Structural determinants of $InsP_3R1$. The rat $InsP_3R1$ (aa 1–2749) can be divided into five fragments resulting from limited trypsin proteolysis [4]. The positions of the four trypsin cleavage sites (346, 924, 1583, 1932) are indicated by arrows. The structures of $InsP_3R_{sup}$ (1–223) [5] and $InsP_3R_{core}$ (224–604) [17] domains are shown above. The $InsP_3R_{core}$ region is color-coded to represent "β-trefoil-type" (yellow) and "armadillo repeat" (green) parts of the structure. Also shown are the Homer-binding site (48–55) [6], the SI alternative splicing site (318–332) [18,19], the TRPC3-binding sites (F2q = 669–702 and F2g = 755–824) [22], the site of SIII splicing (917/918) [21], the LIZ motif (1251–1287) complexed with AKAP9-PKA-PP1-NR1 [24], the Ca^{2+}-CaM binding motif (1565–1586) [25], the PKA/PKG phosphorylation sites (S1589 and S1755) [38–41], the SII alternative splicing site (1692–1731) [18,19,38], the ATPA binding site (1773–1780) [42,43], the site of caspase-3 cleavage (1888–1891) [44], the region deleted in *opisthotonos* mouse mutant (1732–1839) [45], the Ca^{2+} sensor region (1933–2275) [56–58], the E2100 reside critical for Ca^{2+} regulation of $InsP_3R1$ [56,57], the ATPB site (2016–2021), the M1–M6 transmembrane domains (2276–2589), the *N*-glycosylation sites (N2475 and N2503) [61,62], the pore-forming region (2541–2552), the site of association with chromogranins A and B (CGA and CGB) (2550–2569) [70,71], the dimerization domain (2629–2654) [78], the 4.1N-binding site (2627–2676) [74], the cytochrome c-binding site (2621–2636) [80,81], the HAP1A- and Htt^{exp}-binding site (2627–2736) [75], and the PP1α-binding site (2731–2749) [48]. The regions implicated in association with other $InsP_3R1$-binding partners are shown below for CaM (49–81 and 106–128) [8–10], CaBP1 (49–81) [11,12], RACK1 (1–346 and 346–923) [13], IRBIT (224–604) [23], GAPDH (981–1000) [27] and CARP (1387–1647) [26].