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Role of IP₃ receptor signaling in cell functions and diseases

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Abstract IP₃ receptor (IP₃R) was found to release Ca²⁺ from non-mitochondrial store but the exact localization and the mode of action of IP₃ remained a mystery. IP₃R was identified to be P₄₀₀ protein, a protein, which was missing in the cerebellum of ataxic mutant mice lacking Ca²⁺ spikes in Pukinje cells. IP₃R was an IP₃ binding protein and was a Ca²⁺ channel localized on the endoplasmic reticulum. Full-length cDNA of IP₃R type 1 was initially cloned and later two other isoforms of IP₃R (IP₃R type 2 and type 3) were cloned in vertebrates. Interestingly, the phosphorylation sites, splicing sites, associated molecules, IP₃ binding affinity and 5' promoter sequences of each isoform were different. Thus each isoform of IP₃ receptor plays a role as a signaling hub offering a unique platform for matching various functional molecules that determines different trajectories of cell signaling. Because of this distinct role of each isoform of IP₃R, the dysregulation of IP₃ receptor causes various kinds of diseases in human and rodents such as ataxia, vulnerability to neuronal degeneration, heart disease, exocrine secretion deficit, taste perception deficit. Moreover, IP₃ was found not only to release Ca²⁺, but also to release IRBIT (IP₃ receptor binding protein released with inositol trisphosphate) essential for the regulation of acid-base balance, RNA synthesis and ribonucleotide reductase.

The beginnings When cells receive signals through receptors on the plasma membrane, the information is transmitted to various types of signaling cascades inside the cells leading cells to respond. Among the various intracellular signaling pathways, IP₃ signaling and diacylglycerol (DAG)-protein kinase C (PKC) pathways are fundamental to cell physiology. Upon activation of

upstream receptors, two messenger molecules, IP₃ and DAG are hydrolysed from phosphatidylinositol 4,5-bisphosphate (PIP₂). This bifurcating signaling pathway is of fundamental importance in regulating a wide range of cellular processes (Fig. 1). DAG activates PKC to phosphorylate various proteins, leading to various cellular responses. As for the other messenger molecule IP₃, an important breakthrough occurred when it was demonstrated that IP₃ releases Ca²⁺ from non-mitochondrial internal stores (Streb et al. 1983) and that this Ca²⁺ released from internal store seemed to play various roles. It was puzzling why IP₃, a molecule with such simple structure, could exert such diverse functions.

Search for the target molecule of IP₃

Why does IP₃ exert such a variety of phenomena? The source of variety could be the function of Ca²⁺ or IP₃R. There are many papers on the function of Ca²⁺ up to now (Carafoli and Klee 1999; Mikoshiba 2007b; Mikoshiba 2012; Pochet 2000; Signal 1995) (Islam 2012). Therefore, I here focus on the mechanism how IP₃ signal is converted to multiple signals. To understand the function of IP₃, one way is to analyze the enzymes that synthesizes or degrade IP₃. Another way is to study the property of the target molecule of IP₃, IP₃ receptor, and investigate its biochemical, molecular and physiological properties. For this purpose they tried to identify IP₃R protein. There were various approaches to understand the property of IP₃R. One approach is to purify IP₃ binding protein and to subsequently determine of the amino acid sequence. Another approach is to clone the cDNA that encodes the full-length IP₃R, which also tells us the amino acid sequence of the IP₃R. The most important thing was to purify and characterize the IP₃R from the aspects of biochemistry and physiology.

Purification of the IP₃ receptor

Many

researchers were attempting to purify the protein from various tissues (Spat et al. 1986) (Prentki et al. 1984; Sudhof et al. 1991; Supattapone et al. 1988) (Maeda et al. 1988): The IP₃ binding protein was purified from rat cerebellum (Supattapone et al. 1988) (Maeda et al. 1988), bovine cerebellum (Hingorani and Agnew 1991; Hingorani and Agnew 1992), bovine aorta smooth muscle (Chadwick, Saito and Fleischer 1990), rat vas deferens (Mourey et al. 1990) and *Xenopus laevis* oocytes (Parys et al. 1992).

cDNA cloning of the IP₃R

One approach was to screen the cDNA expression library by the antibodies which were specific to the IP₃ receptor. Surprisingly, most successful approach to identify IP₃R protein and raising antibody against it was the analysis of the ataxic mutant cerebellum. During the analysis of the cerebellar ataxic mutant, P₄₀₀ protein, high molecular glyco-phospho-membrane protein was found to be almost missing in the cerebellum of mutant mice such as *pcd* (*Purkinje cell degeneration*), which lacks the cerebellar Purkinje neurons, or *staggerer* whose dendritic arborization is poor and whose spines on the dendrites were completely absent (Maeda et al. 1989; Maeda et al. 1988; Mikoshiba, Huchet and Changeux 1979). Later it was also found that *staggerer* mutant lacked Ca²⁺ spikes in the Purkinje cells in the cerebellum (Crepel, Dupont and Gardette 1984). As long as two years and a half were spent raising the specific monoclonal antibodies against P₄₀₀. Finally P₄₀₀ was identified to be the IP₃R. One important piece of evidence to conclude that P₄₀₀ was indeed IP₃ receptor was the fact that P₄₀₀ monoclonal antibody immunoprecipitated the IP₃ binding activity from the solubilized cerebellar tissues. Expression cloning from the cDNA library of the cerebellum using the specific monoclonal antibodies successfully led them to clone the entire sequence of IP₃R type 1 (IP₃R1). The sequence was about 10 kilo base-pair and encoding a peptide of about 2700 amino acids long. (Furuichi, Yoshikawa and Mikoshiba 1989) (Furuichi et al. 1989) Another group used a different approach using differential subtraction analysis of cerebellar cDNAs between wild-type and the *pcd* (*Purkinje cell deficient*) mutant. The partial sequence (PCD6) obtained by differential subtraction (Nordquist, Kozak and Orr 1988) was found to be different from that of the ryanodine receptor reported by Prof. Numa (Takeshima et al. 1989). The partial sequence was hypothesized to be part of the sequence of the putative IP₃ receptor judged from the sequence homology and its localization at ER (Mignery et al. 1989).

cDNA cloning of IP₃R from various species was successful. There are three isoforms in vertebrates (Furuichi et al. 1994; Mikoshiba 2007a): human (Yamada et al. 1994; Yamamoto-Hino et al. 1994), mouse (Furuichi, Yoshikawa and Mikoshiba 1989; Furuichi et al. 1989; Iwai et al. 2005; Yamada et al. 1994), rat (Mignery et al. 1990) (Südhof et al. 1991), *Xenopus laevis* (Kume et al. 1993) and single isoform in invertebrates (star fish)(Iwasaki et al. 2002), *C. elegans* (Dal Santo et al. 1999), *Drosophila melanogaster* (Hasan and Rosbash 1992; Yoshikawa et al. 1992), *Trypanosoma Cruzi* (Hashimoto et al. 2013) are found.

IP₃R works as a signaling hub for diversified cell function

There are accumulating evidences that IP₃ receptor plays an important role in cell function. Importance of the fact that IP₃R forms macro-molecular complex which works as a signaling hub by association with various molecules and determines its direction of cell signaling pathways is now recognized, which makes it possible for IP₃Rs to produce diversified cell signaling by using simple IP₃ molecule.

1) Unique biochemical and biophysical property of the IP₃R

IP₃ receptor is a large protein with molecular weight 313,000 dalton. 313kilo Dalton protein is a monomer and IP₃ receptor was found to be composed of tetramer (Maeda et al. 1991). Intensive biochemical and biophysical studies were performed by many researchers and it was found that IP₃R has IP₃ binding site located at the N-terminal portion and transmembrane region existed at the C-terminal portion (Foskett et al. 2007; Mikoshiba 2007a) (Patterson, Boehning and Snyder 2004)

It was not clearly demonstrated that IP₃R is a Ca²⁺ channel, since all the data was obtained by using the microsomal fraction which contained endoplasmic reticulum, plasma membrane and other membrane fractions (Bezprozvanny, Watras and Ehrlich 1991). However Purified tetrameric IP₃R was found to be a Ca²⁺ channel in the presence of IP₃ and Ca²⁺ by incorporating a purified IP₃ binding protein into lipid bilayer and liposome (Ferris, Haganir and Snyder 1990; Foskett et al. 2007; Maeda et al. 1991; Mikoshiba 2007a). The overexpression of IP₃ receptor type 1 cDNA enhanced IP₃ binding activity and Ca²⁺ release activity (Miyawaki et al. 1990). Immunohistochemical study showed that it is localized on the endoplasmic reticulum (Otsu et al. 1990) (Satoh et al. 1990; Yamamoto et al. 1991)

At the N-terminal domain of IP₃ binding pocket, there is a unique sequence called suppressor domain which is important for 1) isoform specific suppressing activity for the IP₃ binding activity that produces an isoform specific IP₃ binding affinity and for 2) gating the channel pore of the IP₃R. IP₃R forms tetramer and IP₃R was found to work as an allosteric protein by modulating its structure by Ca²⁺, IP₃, ATP and other modulator proteins. Whole tetrameric IP₃R changes its conformation dynamically observed by electron microscopy (Hamada et al. 2002) (Hamada

and Mikoshiba 2012; Hamada, Terauchi and Mikoshiba 2003) and FRET analysis (Shinohara et al. 2011).

IP₃ receptors form heterotetramer by cross-linking experiment (Monkawa et al. 1995) as well as homotetramer. The size of each isoform of IP₃R seems to be homologous as far as the localization of IP₃ binding site at N-terminal side, channel region at the C-terminal side. But when compared in detail, there are various differences among three isoforms.

Firstly, 5' promoter region of IP₃ receptors are different, and secondly, IP₃ binding affinity of each isoform is different among three isoforms. The thirdly, glycosylation and the phosphorylation site, splicing site is different among the three isoforms.

The difference of 5' promoter sequence of each isoform suggests that each IP₃R isoform are expressed specifically in each type of cells informing us isoform-specific cell expression.

IP₃ binding affinity of IP₃ binding pocket from each isoform was almost the same, suggesting that other region determines IP₃ binding affinity (Iwai et al. 2007). The specific IP₃ binding affinity was found to be regulated by the suppressing activity by N-terminal region, therefore this region was called suppressor domain (SD) (Yoshikawa et al. 1999a).

(Yoshikawa et al. 1999b) (Uchida et al. 2003)

The glycosylation sites and phosphorylation sites may play a role for unique functions of each isoform and the function should be studied furthermore. These differences in the posttranscriptional modification suggest that each isoform of IP₃R binds to many different functional proteins.

2) IP₃R associates with various molecules

Actually IP₃R was found to bind various types of molecules. IP₃R binds glutamate receptor (mGluR, AMPA receptor, NMDA receptor) through cytoskeletal proteins. Cytoskeletal protein, homer binds to the N-terminal part and 4.1N binds to C-terminal part of IP₃R1. GRP78, a typical ER chaperone binds specifically to the third luminal loop of IP₃R1 and interacts and work together to resist to ER-stress. Association of GRP78 to IP₃R1 is decreased by the ER-stress and also in the cells of Huntington disease mouse and also the cells from the patients (Higo et al. 2010). ERp44 is a homologue of thioredoxin family and interacts under reduced condition with the third luminal loop of IP₃R1 and inhibits IP₃ induced Ca²⁺ release (Higo et al. 2005). GIT1 and GIT2 are G-protein-coupled Receptor Kinase-interacting Proteins and associate with the C-terminal tail of all IP₃R isoforms in a Ca²⁺ dependent manner inhibit IP₃R (Zhang et al. 2009).

80K-H interacts with the C-terminal tail of all IP₃Rs and stimulate IP₃R (Kawaai et al. 2009). Cytochrome c interacts with the C-terminal tail of IP₃R1 and 3 and suppresses IP₃R inhibition by high cytosolic Ca²⁺ (Boehning et al. 2003). Huntingtin which is considered to be a causal gene interacts with the C-terminal tail of IP₃R1 and sensitize the IP₃R to IP₃ depending on the polyQ extension and the presence of Huntingtin-associated protein 1 (Tang et al. 2003). Na⁺K⁺ATPase, which is considered to be important for Na⁺, K⁺ balance between the plasma membrane. Na⁺K⁺ATPase binds with the ligand binding domain of all IP₃R isoforms and stimulates IP₃R in the presence of ouabain. (Zhang et al. 2006). Protein phosphatases (PPI and PP2A) are found to be associated with IP₃R1 and also cAMP-dependent protein kinase and these molecules are considered to form macromolecular signaling complex (DeSouza et al. 2002). RACK1 serves as a scaffold, linking protein kinase C to its substrates. RACK1 physiologically binds IP₃ receptors and regulates Ca²⁺ release by enhancing IP₃ receptor binding affinity for IP₃ (Patterson et al. 2004). Ankyrin B interacts with IP₃R in high affinity and this association is important for the IP₃R posttranslational stability (Mohler et al. 2004). Ankyrin B also binds to Na⁺, K⁺ ATPase and Na⁺/Ca²⁺ exchanger 1 and is considered to play a role for cytosolic Ca²⁺ modulation (Mohler, Davis and Bennett 2005). Chromogranin A and B interact in a pH-dependent manner with IP₃R between the pore region and transmembrane 6 and stimulate IP₃R activity (Choe et al. 2004). CARP (carbonic anhydrase-related protein) is specifically expressed in the Purkinje cells in the cerebellum binds to a central part of the molecule between the IP₃-binding core and the channel region and CARP reduces the affinity of IP₃R1 to IP₃ (Hirota et al. 2003). Bcl2 and Bcl-X1 are anti-apoptotic proteins which interact with all IP₃Rs (Chen et al. 2004; Monaco et al. 2012; Rong et al. 2008; White et al. 2005)

3) New signaling molecule, IRBIT, released from IP₃ receptor

The function of IP₃R was considered only to release Ca²⁺ through Ca²⁺ channel. However, it was found that in addition to Ca²⁺ release, IRBIT (IP₃ receptor binding protein released with inositol trisphosphate) (Ando et al. 2006; Ando et al. 2003; Mikoshiba 2007a) was discovered from the analysis of the proteins that associate with IP₃ receptor (Fig. 2). Column with native IP₃ receptor was incubated with the soluble brain fraction and eluted step-wise increase of salt concentration, and finally eluted with the IP₃ with high salt concentration. A single protein was eluted and named as IRBIT (IP₃ receptor binding protein released with inositol trisphosphate) which was a homolog of S-adenosyl homocysteine hydrolase (SAHH) (Ando et al. 2006; Ando et al. 2003)

having phosphorylated site at the N-terminal region of IRBIT with about 50% homology with SAHH. It does not exhibit SAHH activity. IRBIT binds to the IP₃ binding pocket of the IP₃ receptor. Phosphorylated phosphates are required for the binding. And the several amino acids required for the recognition of IP₃ is required for the IP₃ binding and any single mutation results in the absence of binding to the IP₃ binding pocket. Therefore, IRBIT may be considered to be the pseudo-ligand for IP₃. IRBIT worked as a pseudo-ligand and addition of IRBIT results in a decreased Ca²⁺ release.

At a resting state, IRBIT was found to be associated with the IP₃ receptor and when high amount of IP₃ was added IRBIT is eluted. Target molecule of IRBIT was found to be Na⁺, HCO₃⁻ cotransporter 1 (Shirakabe et al. 2006), CFTR (Yang et al. 2009), and Na⁺H⁺ exchanger (Tran et al. 2013). These molecules are found to be important for regulation of pH balance inside the cell. The data suggest that IP₃R regulates pH inside the cell through IRBIT (Ando, Kawaai and Mikoshiba 2014).

IRBIT was reported to bind to Cleavage and Polyadenylation Specificity Factor (CPSF) and regulated polyA addition, thereby IRBIT regulates mRNA synthesis (Kiefer et al. 2009). IRBIT was also reported to be a novel regulator of ribonucleotide reductase in higher eukaryotes (Arnaoutov and Dasso 2014).

Inhibitors of IP₃ receptors

Heparin, 2-aminoethoxydiphenyl borinate (2-APB) (Maruyama et al. 1997) and Xestospongine C (Gafni et al. 1997) are most widely used inhibitors for IP₃Rs. Heparins are sulfated glycosaminoglycans and competitively inhibit IP₃R by binding to the IP₃ binding site. Problems of the heparin are the non-specific effect with other proteins with no permeability to the plasmamembrane.

2-APB is a boron compound and it was discovered by the screening of IP₃ receptor inhibitor. It is a membrane permeable compound. 2-APB was also found to block the store operated Ca²⁺ entry (SOCE) channel activity in addition to IP₃ induced Ca²⁺ releasing activity (Fig. 3: left) (Gregory, Rychkov and Barritt 2001; Iwasaki et al. 2001; Kukkonen, Lund and Akerman 2001; Ma et al. 2001; Prakriya and Lewis 2001) (see detailed review on 2-APB in Cell Calcium 2010 (Goto et al. 2010))

By modifying the 2-APB structure, various compounds were synthesized by organic chemistry, drugs that block the SOCE channel was finally developed as DBP 162AE, DBP163AE after

intensive screening (Goto et al. 2010).

Xestospondin is an extracted compounds and the structure seems similar to 2-APB with two phenol rings (Fig. 3: right) (Gafni et al. 1997). This compound is also membrane permeable and is also used often as 2-APB.

There is still a problem of specificity in both 2-APB and Xestospongine C (Parys and Smedt 2012)

Recently, IP₃ sponge which is made from the IP₃ binding core of IP₃R after some mutations of the amino acid of the IP₃ binding core is used as an IP₃ absorbent (Uchiyama et al. 2002). IP₃ sponge inhibits the sugar taste sensation in *Drosophila* (Usui-Aoki et al. 2005) and blocks fertilization when it is injected to oocytes (Iwasaki et al. 2002). Transgenic mouse which express IP₃ sponge in an astrocyte specific manner attenuated the IP₃ induced Ca²⁺ release. The transgenic mice exhibited behavioral impairments in spatial reference memory and remote contextual fear memory (Tanaka et al. 2013).

By taking good use of these inhibitors, it is possible to study the role of IP₃ induced Ca²⁺ release in cell signaling.

Role of each isoform of IP₃R in cell signaling

As above mentioned, molecules are expressed in a cell type-specific manner and bind to each isoform in an isoform specific manner. Since the size of the IP₃R, especially the site between the IP₃ binding site and transmembrane channel pore region is long, it is plausible to consider that IP₃R offers a platform for various molecules to interact each other. Three different platforms may offer different trajectories of cell signaling. Various disease states occur depending upon which IP₃R subtype and signaling trajectory is abnormal.

IP₃R 1 is essential for Ca²⁺ oscillations in egg and important for fertilization. IP₃R1 determines the fate of abdomen and back at early developmental stages. IP₃R1 is involved in neurite extension and neural plasticity. Absence of IP₃R1 causes abnormal fertilization (Miyazaki et al. 1992) and dorso-ventral axis formation at early stages (Takei et al. 1998). Severe ataxia is caused by IP₃R1 mutations in humans and mice (Matsumoto et al. 1996; Tada, Nishizawa and Osamu 2014).

Blockade of IP₃R1 caused by ER stress and susceptibility to brain damage (Higo et al. 2010).

IP₃R2 is enriched in heart and overexpression in heart results in hypertrophy of cardiac muscle. This cardiac hypertrophy is rescued by introducing IP₃ sponge peptide that trap IP₃ (Nakayama et al. 2010). IP₃R2 mutation in human-being results in anhydrosis (Klar et al. 2014). IP₃R1 and IP₃R3 double knock out mice die due to cardiogenesis arrest at E15 at particular region such as

outflow tract region of heart accompanied with Mef2C–Smyd1 signaling pathway abnormality (Nakazawa et al. 2011). IP₃R1 and IP₃R2 double knock out mice shows defects of the atrioventricular canal and ventricular myocardium of the heart due to the calcineurin/NFAT signaling abnormality. They die at about E15 (Uchida et al. 2010).

The IP₃R3 deficiency results in abnormalities in taste perception (Hisatsune et al. 2007), hair growth cycling (Sato-Miyaoka et al. 2012), and osteoclast formation (Kuroda et al. 2012), and also results in nasal inflammation (Fukuda et al. 2008). Therefore, it is concluded that IP₃R3 is important for taste perception, nasal formation, and hair growth and osteoclast formation. IP₃R2 and IP₃R3 are important for exocrine fluid secretion including saliva, pancreatic juice (Futatsugi et al. 2005) and tear secretion (Inaba et al. 2014). IP₃R2 and IP₃R3 double knockout results in dry eye and dry mouth and pancreatic juice secretion deficit (Futatsugi et al. 2005). These phenotypes are those of Sjögren Syndrome which is an autoimmune diseases. The antisera from autoimmune disease patients were collected and analysed and were found that antisera from autoimmune patients contain anti-IP₃R antibodies (Miyachi et al. 2009). Therefore, detection of the antibody against IP₃R in the sera from the candidate of autoimmune disease patients could be considered to be a good candidate for the one of the diagnostic tool of autoimmune disease.

Evidently, each IP₃R type plays a specific role as a signaling hub by association with various molecules and this in turn determines its trajectory of cell signaling and function (Fig. 4). The detailed mechanisms how these delicate differences should be further analyzed, but it is clear that each IP₃R produces diversified cell signaling paths from a single IP₃R molecule. Therefore, when there occurs an abnormality in IP₃ receptor, abnormal cell function occurs resulting in severe diseases (Berridge 1998; Foscett 2010; Hara and Snyder 2007; Mikoshiba 2007a; Patterson, Boehning and Snyder 2004).

cDNA cloning of *Trypanosoma Cruzi* IP₃ receptor, and detection of Ca²⁺ oscillation in Malaria (*Plasmodium Falciparum*) worm

Analysis of primitive living organisms sometimes demonstrates us the essence of life and principle of the phenomena.

cDNA of IP₃ receptor of *Trypanosoma Cruzi* was cloned and sequenced. It was a single gene. Knock down or overexpression of the receptor resulted in death of the worm suggesting delicate amount of IP₃ receptor is essential for the worm for surviving (Hashimoto et al. 2013). Since the

IP₃ receptor of *Trypanosoma Cruzi* is different from other species including human-being, antisense oligonucleotides strategy targeting parasite IP₃ receptor works quite efficiently to inhibit mammalian host cell invasion by the worm (Hashimoto et al. 2013).

The spontaneous Ca²⁺ oscillation of the parasite of *Plasmodium Falciparum* was observed in the intraerythrocytic *Plasmodium Falciparum*, and 2-APB blocked the Ca²⁺ oscillation and resulted in the death of the worm suggesting Ca²⁺ oscillation is essential for the *Plasmodium Falciparum* for the survival of the worm (Enomoto et al. 2012).

In summary

IP₃ receptor is now considered to be an important signaling molecule. Biochemical and molecular biological studies including the primary amino acid sequence and three dimensional structure helped us to understand the mechanism of IP₃ receptor that exerts multiple functions. It is found from the various evidences that IP₃ receptor is working as a signaling hub for the cell signaling. Each type of IP₃ receptor has its own signaling hub that determines different trajectories of cell signaling resulting a variety of cell functions assisted by calcium oscillation. Dysregulation of IP₃ receptor causes severe diseases in human and rodents.

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Fig 1

IP₃- IP₃ receptor and Diacylglycerol (DAG)-Protein Kinase C (PKC) signaling system.

IP₃ binds to IP₃ receptor and releases Ca²⁺ from intracellular stores and also releases IRBIT from the IP₃ binding core of IP₃ receptor. DAG activates PKC and PKC regulates various molecules by phosphorylation.

Fig. 2

IRBIT(IP₃R binding protein released with inositol 1, 4, 5-trisphosphate) is a pseudo-ligand of IP₃ receptor.

IRBIT binds to the IP₃ binding core and released in the presence of high concentration of IP₃. The amino acids necessary for the recognition of IP₃ are also necessary for the recognition of

IRBIT (Ando et al. 2006; Ando et al. 2003). Phosphorylation of IRBIT is required to be recognized by the IP₃ R. IRBIT suppresses IP₃ induced Ca²⁺ release but when IRBIT is treated by siRNA, amplitude and frequency of Ca²⁺ oscillation were enhanced.

Fig. 3

Structure of 2-APB (left) and Xestospongine C (right) which are IP₃ receptor inhibitor

Fig. 4 Each IP₃R has each different signaling hub for the cell regulation assisted by calcium oscillation.

Three isoforms of IP₃R determine the trajectory for cell signaling inside the cell. : Three types of IP₃R cDNA are cloned and the whole sequences are determined in vertebrate (mouse, rat and human-being). There are various structural differences in phosphorylation sites, glycosylation sites, splicing sites and also the sequence itself. In addition, each isoform has different IP₃ binding affinity. 5' promoter region of cDNA of each isoform is different suggesting that the each isoform has its cell type specific expression pattern. As is well described in the text (see the section of "IP₃R works as a signaling hub for diversified cell function") each isoform forms macro-molecular complex which works as a signaling hub by association with various molecules and determines the direction of cell signaling pathways. Various diseases occur depending upon which IP₃R is abnormal.

Traditional concept is that the Ca²⁺ determines all the various functions based on the Ca²⁺ oscillation. According to the novel concept, each isoform among three isoforms determine the different trajectory of cell signaling assisted with the Ca²⁺ signaling including Ca²⁺ oscillation.

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Figure 1

Figure 2

Figure 3

Figure 4