CMLS, Cell. Mol. Life Sci. 56 (1999) 296–304
1420-682X/99/040296-09 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Role of the inositol 1,4,5-trisphosphate receptor in early embryonic development

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Received 9 February 1999; received after revision 15 April 1999; accepted 7 May 1999

Abstract. There is now considerable literature on the importance of phosphatidylinositol cycle activation in transducing information of various types across the plasma membrane. Though much of the data derives from studies on somatic cells, there is increasing evidence for crucial events related to development, including fertilization, cell cycle progression and dorsoventral axis formation. In this review, focus is directed mainly to the molecular basis of the inositol 1,4,5-triphosphate receptor expressed in oocytes and early embryos of *Xenopus*. Recent progress in studies concerning the role of this receptor in early embryonic development is discussed.

Key words. IP_3; Ca^{2+}; dorsoventral axis formation; fertilization; development.

Introduction

In many species, when sperm attach to eggs, a drastic calcium (Ca^{2+}) transient, the so-called Ca^{2+} wave, occurs at the attachment site and propagates as a wave to the opposite side across the entire egg. Later, when the one-cell embryo is about to divide, a spontaneous Ca^{2+} transient is again observed [1]. When the dorsoventral axis is about to be specified, a transient increase in IP_3 mass is detected during the 32–64-cell stage embryos of *Xenopus* [2]. The role of Ca^{2+} in embryonic development has fascinated many researchers. The 'inositol depletion hypothesis' has been proposed to explain the teratogenic effects of lithium, suggesting that the phosphatidylinositol (PI) cycle might be the target of lithium. While many efforts have been made, the mechanism of the teratogenic effects of lithium has not entirely been clarified. There is now clear evidence for an important role for IP_3-Ca^{2+} signaling in regulating fertilization, cell cycle progression and dorsoventral axis formation. The molecular basis and expression pattern of the IP_3 receptor in eggs and early embryos are reviewed herein, and recent insights into the role of IP_3-Ca^{2+} signaling in early embryonic development are addressed.

The IP_3 receptor

In mammalian somatic cells, activation of PI signaling triggers hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) by phospholipase C-β or -γ, producing inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) [3]. IP_3 is the key second messenger that mediates cellular functions in a variety of cells by mobilizing Ca^{2+} from intracellular stores, mainly the endoplasmic reticulum (ER), into the cytosol through the IP_3 receptor (IP3R) (fig. 1). IP3R was originally purified from the rodent cerebellum [4–6], and subcellularly localized mostly at the ER [7–9]. IP3R complementary DNA (cDNA) was isolated, and its primary structure was determined to be an IP_3-gated Ca^{2+} channel (see [10–

12] for review). In addition to type 1 IP3R, the best characterized and the predominant type in the brain, different types of IP3R derived from different genes have been molecularly cloned, and differences in IP_3-binding and Ca^{2+} release activity of each receptor type have been determined. The existence of differential IP_3-Ca^{2+} signaling in the three receptor types was considered [12–14]. Although IP3R is concentrated mostly in cerebellar tissue, particularly in Purkinje cells [15, 16], studies of the expression pattern of IP3R in mice revealed it to be expressed in various tissues, including mature ovarian oocytes [15, 17]. This suggests the functional importance of IP3R in various cell types or tissues.

Dynamism of IP3R-Ca^{2+} pools

IP3R is localized in the ER, which serves as an internal pool for Ca^{2+} storage and extends throughout the cell. The ER network appears to be a continuous membrane system, as noted when monitoring the diffusion of a fluorescent, lipophilic dye, in neuronal cells and *Xenopus* oocytes or mouse eggs [18–20]. The ER and cytoskeleton seem to be interdependent structures [21]. The IP3R-Ca^{2+} pools may interact with the cytoskeleton [22], which in turn may have a role in subcellular anchoring or the dynamics of IP3R-Ca^{2+} pools. The IP3R-Ca^{2+} pools are highly dynamic structures capable of changing structure and subcellular localization during meiotic maturation and fertilization. During the event of meiotic maturation, IP3R-positive Ca^{2+} pools undergo dynamic relocalization from the cytoplasm to the cortical region in mouse and *Xenopus* oocytes [19, 20, 23, 24]. The structural difference of the ER in oocytes and eggs correlates well with an increase in the mobility of the ER [19], as well as acquisition of the IP_3-induced Ca^{2+} release (IICR) mechanism [19, 24–28], as shown by the observations that IICR is much higher in mature eggs than in immature oocytes and that the amount of IP3R protein increases in the cortical region after meiotic maturation of the mouse oocytes [24]. Since immature oocytes possess an amount of releasable Ca^{2+} stores similar to mature eggs [25], meiotic mature eggs might first become activation-competent through reorganization of IP3R-Ca^{2+} pools. Besides the predominantly expressed type 1 IP3R, type 2 and 3 IP3R are also shown to be spatially and biochemically heterogeneous. It remains an open question whether the spatial heterogeneity of IP3R has a role to play in conferring a mechanism for propagating Ca^{2+} release from the cortex into the interior of the egg to activate development [29]. At fertilization, a similar dynamism of IP3R-Ca^{2+} pools was observed in the cortical region of eggs of sea urchin, *Xenopus* and starfish [23, 30–32].

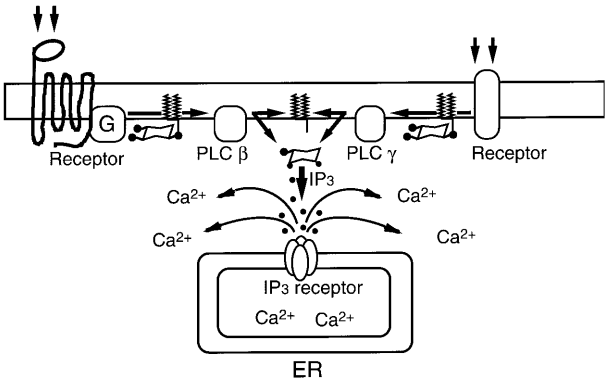

Figure 1. Scheme of IP_3-Ca^{2+} signaling transduction cascades. Activation of IP_3-$Ca2^{+}$ signaling is triggered by at least two mechanisms: either by binding of the neurotransmitters, neuropeptides and so on to specific receptors (seven-membrane spanning receptors) coupled with guanosine 5′-triphosphate (GTP)-binding protein (G), or otherwise by binding of growth factors to their specific receptors possessing tyrosine kinase activity. In the former cascade, phospholipase C-β1 is activated and catalyzes the breakdown of PIP_2, producing IP_3 and diacylglycerol. IP_3, in turn, triggers Ca^{2+} release through IP3R. In the latter cascade, receptor tyrosine kinase then activates PLCγ, which triggers breakdown of PIP_2, and activation of the protein tyrosine kinase family.

IP_3-Ca^{2+} signaling and egg activation

The unfertilized eggs of many species are quiescent, and some resemble growth-arrested somatic cells in that their cell cycles are arrested. Following fertilization, however, the egg is transformed into an active and rapidly proliferating cell system.

A transient increase in intracellular Ca^{2+} concentration has been observed during the activation of a wide variety of eggs [33–38]. At the time of fertilization there is an increase in IP_3, and microinjection of IP_3 into eggs of the frog, sea urchin, medaka, starfish and hamster mimicked some early developmental events of egg activation, such as membrane depolarization, cortical granule exocytosis, cortical contraction, pronuclear formation, emission of the polar body and inabortive cleavage furrow formation [39–44]. It has been proposed that the binding of a sperm to its hypothetical receptor at the egg surface activates PLC [45, 46], and release IP_3, which stimulates the release of Ca^{2+} from intracellular stores [43, 47–49]. The ryanodine receptor (RyR) was also identified as a Ca^{2+} channel involved in the release of free Ca^{2+} from the ER [50]. While IP3R is responsible for IICR, RyR is responsible for Ca^{2+}-induced Ca^{2+} release (CICR). In *Xenopus*, mouse and

hamster eggs, Ca^{2+} is released through IP3R [23, 51–53], whereas in other species such as sea urchin, it is released through both IP3R and RyR [54–56, 57 for review]. It has been demonstrated that injection of an IICR-blocking antibody against mouse IP3R inhibited both the initiation and the propagation of Ca^{2+} waves in hamster eggs [58], as well as some early and late events of activation, such as sperm-induced modifications of the zona pellucida (ZP), fertilization-associated decrease in H1 kinase activity, emission of the second polar body and pronucleus formation, recruitment of maternal messenger RNAs (mRNAs) and initiation of posttranslational protein modifications in mouse eggs [59]. New synthesis of IP3R in *Xenopus* eggs, using sequence-specific antisense oligonucleotides against *Xenopus* IP3R inhibited the IP_3-responsive cortical contraction [23]. Injection of heparin, an IP3R antagonist, into *Xenopus* eggs, inhibited both the occurrence and propagation of Ca^{2+} waves. In the sea urchin, where both IP3R and RyR are present, injection of both heparin and ruthenium red blocked the occurrence and propagation of Ca^{2+} waves [55]. These results mean that IICR plays an important role in initiation and propagation of Ca^{2+} wave upon egg activation.

IP_3-Ca^{2+} signaling associated with cell cycle progression and early embryonic development

After the large Ca^{2+} transient accompanying fertilization, the egg is activated and development begins. As the embryo divides rapidly, Ca^{2+} is thought to participate in regulating several aspects of cell division [60, 61 for review], as well as body patterning in early embryonic development. There are several lines of evidence suggesting a close correlation between the IP_3-Ca^{2+} signaling system and progression of the cell cycle in embryos of various species. Cyclic changes in Ca^{2+} [62–64] and changes in IP_3 and PIP_2 [65, 66] have been observed in the cleaving *Xenopus* and sea urchin embryos. Intracellular calcium transients are observed at various stages during cell division such as pronuclear migration and fusion, nuclear membrane breakdown, the metaphase-anaphase transition and cytokinesis in sea urchin embryos [66–69], just before nuclear envelope breakdown in the mouse embryo [70] and at cytokinesis of medaka fish or *Xenopus* embryos [64, 71]. Injection of IP_3 stimulates premature nuclear membrane breakdown and chromatin condensation [72]. The IP_3 antagonist heparin blocks both calcium transients and entry into mitosis [64, 66]. The cell cycle in *Xenopus* and sea urchin has been blocked or is greatly lengthened by diminishing Ca^{2+} gradients using Ca^{2+} buffers [73], treatment with antibodies that reduce PIP_2 hydrolysis [65] or injection of lithium, which could be rescued by *myo*-inositol (an intermediate of the PI cycle) [74].

During early embryonic development, measurements of concentrations of the second messenger IP_3 in *Xenopus* revealed that a transient increase in the IP_3 mass occurs in 32–64-cell stage embryos. Later in development, an increase in IP_3 mass in the ectoderm occurs after the early gastrula stage 10.5, and this parallels localization of the *Xenopus* IP3R protein in this region [75]. In zebrafish embryos, two distinct phases of dramatic Ca^{2+} signaling events have been identified: one is a long-lived elevation of intracellular free Ca^{2+} localized to forming cleavage furrows, accompanying the first few cell divisions [76]; another is that by the 16–32-cell stage, cells of the enveloping layer of the blastodisc begin to display rapid and periodic Ca^{2+} transients [77]. Whether there is a dorsoventral gradient in the occurrence of Ca^{2+} transient in early embryos remains to be determined.

Localization of IP3R in early embryos

IP3R is expressed as a maternal protein during early stages of cleavage, at a substantial level [40, 75, 78]. In addition to its predominant localization on ER, IP3R is densely localized in the perinuclear region in *Xenopus* oocytes or embryos [23, 75]. There are data suggesting that a functional PI signaling system is present in the nuclei [79, 80]: in early sea urchin embryos, IP_3-Ca^{2+} signaling may be involved in controlling chromosome dysjunction [68], and nuclear IP3R may be involved in the fusion of postmitotic nuclear membranes [81].

There is no apparent dorsoventral gradient in the expression of *Xenopus* IP3R protein, as determined by immunohistochemical analysis [S. Kume et al., unpublished results]. Although the intracellular machinery for transducing dorsal or ventral signals may be evenly distributed, a possible spontaneous transient increase in the level of IP_3 may occur, possibly forming a gradient ranging from low levels on the dorsal side to high levels on the ventral side (fig. 2). Such a gradient of signaling activity could be attributed to localization of ligand molecules or cell surface receptors that modulate IP_3-Ca^{2+} signaling. It will be of interest to determine whether dorsoventral gradient of an IP_3-Ca^{2+} signaling activity exists in response to the upstream ligand of this signal cascade.

IP_3-Ca^{2+} signaling as a ventralizing signal

The pattern formation of the body plan of *Xenopus laevis* has been suggested to involve a sequence of inductive events: mesoderm and neural inductions, which result in regional specification of cells. Although the molecular basis of these mechanisms is not well understood, it is likely that receptor-mediated signal

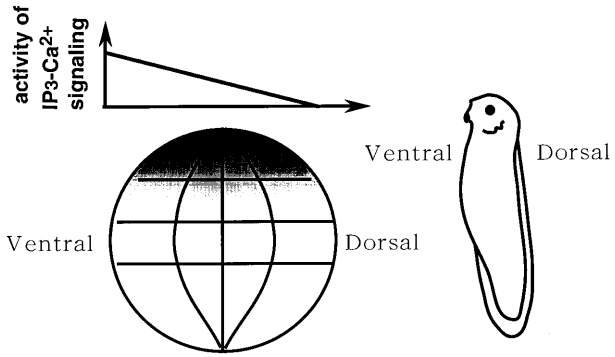

Figure 2. A working hypothesis for the dorsoventral gradient of the activity of IP_3-Ca^{2+} signaling. It may be that there is a spontaneous transient increase in the level of IP_3, possibly forming a gradient ranging from low level on the dorsal side to high levels on the ventral side. Such a gradient of signaling activity may relate to the possible localization of ligand molecules or cell surface receptors that finally modulate IP_3-Ca^{2+} signaling. It will be of interest to determine whether dorsoventral gradient of IP_3-Ca^{2+} signaling activity exists in response to the upstream ligand of this signaling cascade.

transduction processes play a key role. Efforts have been made to identify molecules and their receptors that mediate these inductive events.

The PI cycle had long been postulated to have a role in the dorsoventral axis formation, as implicated by the action of lithium chloride in many species: early application (cleavage stage) of lithium to *Xenopus* embryos induces dorsalization and causes a reduction of posterior structures [82 for review, 83–88], whereas a late application (gastrula stage) causes ventralization, which results in reduction of anterior structures [89, 90]. Lithium is assumed to act by inhibiting several key enzymes, such as inositol 1-phosphatase and inositol monophosphatase, which are responsible for hydrolysis of intermediate inositol phosphates, thereby depleting the supply of inositol [82 for review]. There are several lines of evidence suggesting that PI signaling is a target candidate of lithium and plays a role in patterning the body axis. The early lithium phenotype could be rescued by coinjection of *myo*-inositol with lithium, but not by the coinjection of *epi*-inositol (an isomer not part of the PI cycle) [91, 92 for review], indicating that the inositol depletion hypothesis may provide a plausible explanation for the effect of lithium. There is an increase in total embryonic IP_3 mass during the early blastula stage of *Xenopus* embryos, as described above. However, the IP_3 mass decreases after lithium treatment [2].

There is also evidence which does not support the inositol depletion hypothesis to interpret the action of lithium: the action of lithium upon *Xenopus* dorsoventral patterning was not mimicked by an inhibitor, bisphosphonate L-690,330 [93], which is a more potent antagonist of inositol monophosphatase than lithium, one of the target enzymes. Furthermore, lithium inhibited glycogen synthase kinase-3β (GSK-3β) [94], which regulates cell fate determination in diverse organisms including *Dictyostelium* [95], *Drosophila* [96], and *Xenopus* [97].

Gain of function studies by the overexpression of receptors that activate the PI cycle signal in the dorsal part of the embryos led to dorsoanterior-deficient embryos [98]. These findings agree with the hypothesis that the locally elevated activity of the PI cycle blocks dorsoanterior determination, whereas low activity in this pathway can lead to dorsalization. Loss of function can be achieved by injection of inhibitory monoclonal antibodies against *Xenopus* IP3R which block IICR. Ventral injection of these blocking antibodies induced dorsal development and gave rise to duplication of the body axis [99] (fig. 3). Interestingly, the inhibitory effect of antibodies correlated well with their ectopic axis-inducing activities. Such evidence is interpreted to mean that active IP_3 signaling plays an essential role in transducing ventral signals during the process of mesoderm induction.

Data obtained from *Xenopus* IP3R-specific blocking moloclonal antibodies (mAbs) showed that the inhibition of IP3R-mediated IICR in *Xenopus* embryos partially mimicked the effect of lithium. Inhibition of IICR in the ventral part of the embryos induced development of an ectopic dorsal axis, through respecifying ventral to that of dorsal [99]. However, the extent of dorsalization differs between the effect of lithium and that of anti-*Xenopus* IP3R mAbs. Lithium treatment causes a duplication of a complete secondary axis, that is both head and trunk organizer, whereas anti-*Xenopus* IP3R mAb injection induces only a trunk organizer. Lithium can completely rescue dorsal structures in ultraviolet (UV)-irradiated ventralized embryos, whereas only a partial rescue occurs with anti-*Xenopus* IP3R mAbs. Another difference between the effect of lithium and anti-*Xenopus* IP3R mAbs is that lithium but not anti-*Xenopus* IP3R mAbs can sensitize the response of animal cap cells to basic fibroblast growth factor (bFGF). These apparent differences between the action of anti-*Xenopus* IP3R mAbs and lithium may relate to the combination of the inhibition of both the PI cycle and GSK-3β.

Molecules involved in the IP_3-Ca^{2+} signaling cascade

As for the upstream ligand of IP_3-Ca^{2+} signaling during dorsoventral axis formation, a protein in the Wnt family is one putative candidate. Wnt genes encode a family of secreted glycoproteins. These are functionally

distinct Wnt proteins, as determined by their differing abilities to transform cells and by differences in embryonic responses to ectopic wnt signals. *Xwnt-5A*, but not *Xwnt-8* was shown to enhance Ca^{2+} signaling in zebrafish embryos [100, 101]. Overexpression of *Xwnt-5A* with rat *frizzled-2* (*Rfz-2*) increased the frequencies of Ca^{2+} spikes. Interestingly, the Xwnt-5A class does not induce ectopic dorsal axis duplication, yet it does decrease cell adhesion and perturb morphogenetic movement during gastrulation in *Xenopus* embryos. The Wnt-5A class can function in a cell nonautonomous manner to block the ability of members of the Wnt-1 class to induce a secondary axis [102]. There is evidence that distinct wnts elicit distinct responses in the same tissues using different signal transduction pathways [103]. It will be of interest to determine how second messenger systems can be modulated by functionally distinct Wnts or members of the *frizzled* gene family, and whether Xwnt-5A is the endogenous upstream ligand which activates IP_3-Ca^{2+} signaling during embryonic development.

Downstream targets of IP_3-Ca^{2+} signaling

The transduction of many cellular stimuli results in oscillations or in elevation of intracellular concentra-

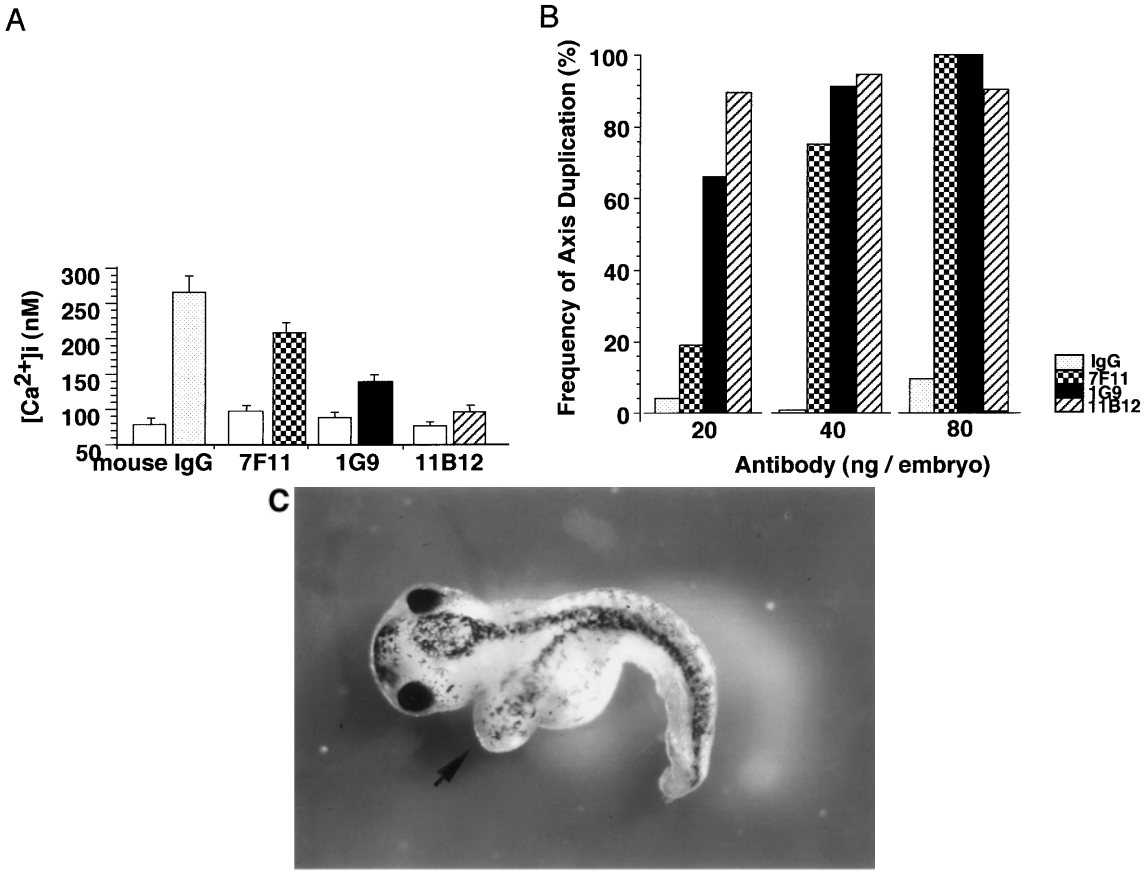

Figure 3. Injection of anti-*Xenopus* IP3R inhibitory antibodies into ventral part of early embryos of *Xenopus* induces an ectopic dorsal axis. (*A*) Effects of anti-XIP3R monoclonal antibodies on IICR in vivo were assessed by their potential to inhibit ligand-gated calcium release in PI-coupled muscarinic acetylcholine receptor type I (m1AChR). In *Xenopus* intact embryos, the potency of the monoclonal antibodies to inhibit ligand (carbachol)-gated IICR was in the following order: 1G9 ≈ 11B12 > 7F11. Ca^{2+} indicator dye Fura 2 (20 μM), m1AChR (13 ng/embryo) and monoclonal antibodies (80 ng per embryo, estimated final concentration of 40 μg/ml) were sequentially injected at the two-cell stage. Animal caps were cut off between the 32-cell and 128-cell stage, and were assayed for ligand-induced Ca^{2+} release. Open bars, before ligand (carbachol, 100 μM) application; closed bars, after ligand application. (*B*) The relative potency of the ectopic axis-inducing activity of the monoclonal antibodies corresponded well to their ability to block IICR: 11B12 ≈ 1G9 > 7F11. (*C*) Ventral injection of anti-*Xenopus* IP3R monoclonal antibody at the four-cell stage induced the formation of a secondary dorsal axis (arrow) in *Xenopus* embryos. Reprinted with permission from: Kume S., Muto A., Suga K., Inoue T., Okano H., Mikoshiba K. (1997) Role of inositol 1,4,5-trisphosphate receptor in ventral signaling in *Xenopus* embryos. Science **278:** 1940–1943, © 1999 American Association for the Advancement of Science..

tions of Ca^{2+}. The molecular mechanism and the downstream targets of the Ca^{2+} oscillations or Ca^{2+} waves observed at fertilization, cell cycle progression or early embryonic axis formation remains largely unknown. There is evidence that varying the frequency or intensity of Ca^{2+} transients can alter the physiological output [104 for review]. One well-known example of molecules modulated by frequency of Ca^{2+} is calmodulin-dependent kinase II (CaMKII), which regulates other enzymes dependent on Ca^{2+}. The enzyme is activated to varying degrees depending on the frequency of Ca^{2+} oscillations [105]. It has been shown that the same total amount of IP_3 analogue elicited much more gene expression when released at a certain interval than when released below or above that interval, as a single pulse or as a slow sustained plateau [106]. It has also been shown that cells are sensitive to modest changes in the concentration of Ca^{2+}, and that different transcription factors can be selectively activated by varying the intensity of Ca^{2+} signals [107]. For example, in B lymphocytes, JNK and NFκB are selectively activated by a large transient Ca^{2+} rise, whereas the transcription factor NFAT (nuclear factor of activated T cells) is activated by a low, sustained Ca^{2+} plateau. Therefore, varying the frequency or intensity of the Ca^{2+} rise can contribute to activation of different subsets of developmental genes. These characteristics of IP_3-Ca^{2+} signaling mean that it is feasible to regulate different developmental programs such as events of fertilization, cell proliferation, dorsoventral axis formation or even other physiological events such as T cell activation, neuronal excitation, neuronal cell migration or synaptic plasticity. Examination of the types of IP_3-Ca^{2+} signaling which activate subsets of genes, which in turn lead to a specific developmental program, is expected to elucidate many of these related events.

Conclusions

Over the past years, much progress has been made in the molecular characterization of IP3Rs and their physiological role in various cell types of tissues. The diversity of IP3Rs and the dynamism of IP3R-Ca^{2+} pools suggest that a highly complicated Ca^{2+} signaling system is finely tuned to specific physiological functions. The question of the upstream ligands and downstream targets of this signaling cascade, and the mechanisms of regulation, particularly at egg activation, cell cycle progression and early body axis formation, remain to be elucidated.

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