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Seminars in Cell & Developmental Biology 17 (2006) 285–292

seminars in
CELL & DEVELOPMENTAL
BIOLOGY

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Review

Signal transduction pathways leading to Ca^{2+} release in a vertebrate model system: Lessons from *Xenopus* eggs

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Available online 2 March 2006

Abstract

At fertilization, eggs unite with sperm to initiate developmental programs that give rise to development of the embryo. Defining the molecular mechanism of this fundamental process at the beginning of life has been a key question in cell and developmental biology. In this review, we examine sperm-induced signal transduction events that lead to release of intracellular Ca^{2+}, a pivotal trigger of developmental activation, during fertilization in *Xenopus laevis*. Recent data demonstrate that metabolism of inositol 1,4,5-trisphosphate (IP_3), a second messenger for Ca^{2+} release, is carefully regulated and involves phospholipase C (PLC) and the tyrosine kinase Src. Roles of other potential regulators in this pathway, such as phosphatidylinositol 3-kinase, heterotrimeric GTP-binding protein, phospholipase D (PLD) and phosphatidic acid (PA) are also discussed. Finally, we address roles of egg lipid/membrane microdomains or 'rafts' as a platform for the sperm–egg membrane interaction and subsequent signaling events of egg activation.

Keywords: Egg activation; Fertilization; Lipid/membrane rafts; Tyrosine phosphorylation; Signal transduction

Contents

1. Introduction

Fertilization involves a species-specific recognition and fusion of egg and sperm, to combine parental genomes into that of a new individual. Major goals in the study of fertilization are to understand the molecular mechanisms of the egg–sperm recognition, binding and fusion, and the associated signal transduction events that culminate in the induction of embryonic development [1,2]. More recently, much attention has been focused on transmembrane signaling cascades involving sperm receptor(s) on egg plasma membranes, molecular switches believed

Abbreviations: $[Ca^{2+}]_i$, intracellular-free Ca^{2+} concentration; IP_3, inositol 1,4,5-trisphosphate; IP_4, inositol 1,3,4,5-tetrakisphosphate; PLC, phospholipase; PI, phosphatidylinositol; PIP_2, phosphatidylinositol 4,5-bisphosphate; DG, diacylglycerol; PKC, protein kinase C; SH2, Src homology 2; PLD, phospholipase D; PA, phosphatidic acid; PH, pleckstrin homology; UPIII, uroplakin III; UPIb, uroplakin Ib; IICR, IP_3-induced Ca^{2+} release; CICR, Ca^{2+}-induced Ca^{2+} release; PIP_3, phosphatidylinositol 3,4,5-trisphosphate; Gαβγ, trimeric GTP-binding protein

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doi:10.1016/j.semcdb.2006.02.008

to transduce the extracellular egg–sperm binding/fusion signal into the intracellular space, and cytoplasmic components involved in the reception as well as propagation of the fertilization signals. This paper reviews recent evidence concerning the sperm-induced egg activation during fertilization in *Xenopus laevis*. We emphasize the roles of the second messengers produced, the enzymes involved in their production such as phospholipases and tyrosine kinase Src, and the egg membrane rafts during fertilization. A series of events for sperm-induced egg activation has been revealed by both in vivo egg experiments and in vitro cell-free approaches. We refer the reader to other references [3–7] for discussion of more general events of *Xenopus* fertilization that are not emphasized here, and for discussion of fertilization in other animals, as well as plant systems, see other papers in this issue.

2. IP_3 metabolism and Ca^{2+} release at fertilization

A sperm-induced elevation of intracellular-free Ca^{2+} concentration ($[Ca^{2+}]_i$)[1] is central to the induction of fertilization events in *Xenopus laevis* and all other species examined [1,8–18]. The mechanism of the initial rise in $[Ca^{2+}]_i$ at the sperm-binding site is controversial and may vary from species to species. A number of studies, however, have confirmed the general importance of the second messenger inositol 1,4,5-trisphosphate (IP_3) as a trigger of the initial rise in $[Ca^{2+}]_i$ [8–16]. Phospholipase C (PLC) hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) to IP_3 and *sn*-1,2-diacylglycerol (DG). IP_3 binds to its receptors [19,20] located in the endoplasmic reticulum to gate this Ca^{2+} channel and allow flooding of Ca^{2+} into the cytoplasm. On the other hand, DG is believed to activate protein kinase C (PKC) [21], which might be responsible for cortical granule exocytosis, cortical contraction, sperm chromatin decondensation, and reformation of the nuclear envelope [3,22,23].

The immature *Xenopus* oocyte undergoes maturation by the action of the steroid hormone to become the fertilizable egg [24]. During maturation, the oocyte moves from the prophase of the first meiosis to metaphase of second meiosis and acquires a higher sensitivity to IP_3 to promote a robust and propagative Ca^{2+} release at fertilization [25]. At fertilization, the increase in IP_3 mass is estimated to be ~200 fmol/egg [26,27] and it is associated with an increase in $[Ca^{2+}]_i$ from ~200–400 nM to ~1.2–1.4 μM [5,28]. IP_3 increases about four-fold over basal levels and peaks at ~5 min after insemination [26,27,29,30].

A $[Ca^{2+}]_i$ wave travels from the sperm-binding site to the opposite side of the egg [28,30–33]. Using a unique rapid sampling and biosensor system, peak IP_3 concentration has been estimated to be 175–430 nM (average of 190 nM) near the sperm entry point and 120–700 nM (average of 370 nM) at half way around the egg from the sperm entry point (value taken just after the Ca^{2+} wave passed that point) [18]. Models for the Ca^{2+} wave involve Ca^{2+} stimulation of Ca^{2+} release [34–36], whereas others suggest a wave of PLC activation [18,37]. In support of the PLC model, a wave of PKC activation, a downstream event of PLC activation, has also been found to be associated with the Ca^{2+} wave [38]. PIP_2 antibodies also decrease, but do not eliminate, the $[Ca^{2+}]_i$ wave [32]. It is also shown that an antibody against the type I receptor for IP_3 is capable of inhibiting Ca^{2+} release in fertilized *Xenopus* eggs [39]. The self-propagating Ca^{2+} wave is believed to be responsible for several biochemical and cellular events of egg activation [22,40,41]. Kline [40] noted that the fertilization membrane depolarization potential (especially sensitive to $[Ca^{2+}]_i$ localized at the plasma membrane), exocytosis of cortical granules, elevation of the fertilization envelope, sperm decondensation and pronuclear formation were completely inhibited by prior microinjection of the Ca^{2+} chelator *O*,*O*′-bis(2-aminophenyl)ethyleneglycol-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA).

Buffering $[Ca^{2+}]_i$ with BAPTA resulted in an elevation of the peak IP_3 value achieved at fertilization, and the time required to attain the peak increased from ~5 to 7 min [27]. From these data, one important function of the increase in $[Ca^{2+}]_i$ at fertilization may be to initiate a negative feedback loop to help turn off the release of Ca^{2+} through stimulation of IP_3 metabolism. In support of this assumption, ^{3}H-labeled IP_3 microinjected into *Xenopus* eggs showed a relatively short half life of ~1 min (Ciapa and Stith, unpublished results) whereas, in the presence of BAPTA, the half life increased to 10 min. By viewing peak sizes of various inositol phosphate(s) in a series of samples collected at various times after ^{3}H-IP_3 injection, the pathway of degradation in the IP_3-activated egg was: IP_3 → inositol 1,3,4,5-tetrakisphosphate (IP_4) → inositol 1,4-bisphosphate → inositol 1-phosphate → myo-inositol (Ciapa and Stith, unpublished results). Thus, it is suggested that an inositol 3′-kinase acts at fertilization to produce the IP_4. Half maximal release of Ca^{2+} is achieved at 88 nM IP_3 whereas that for IP_4 is 3.44 μM [42]. Thus, although IP_4 may have other functions, IP_3 metabolism to IP_4 may turn off the Ca^{2+} release signal. It is interesting to note that this type of metabolic pathway is also known to be present in mammalian cells [43].

3. Regulation of phospholipase C: how is IP_3 production regulated?

Since IP_3 is believed to be the trigger for sperm-induced initial $[Ca^{2+}]_i$ release, the mechanism of the activation of the enzyme that produces IP_3, PLC, has been of particular interest. As noted, even while blocking $[Ca^{2+}]_i$-dependent fertilization events (gravitational rotation, contraction wave, cleavage) with either BAPTA or heparin, an IP_3 receptor blocker, sperm still stimulate PLC and elevate IP_3 and DG [27,44]. Thus, there is a support for the model that sperm do not have to elevate $[Ca^{2+}]_i$ to stimulate PLC. In fact, Ca^{2+} ionophore increased egg IP_3 and DG to values only ~20–30% as large as that induced by sperm [26,44]. We suggest that the initial sperm-dependent activation of PLC does not require elevated $[Ca^{2+}]_i$, however, subsequent stimulation of PLC for Ca^{2+} wave may be due at least in part to the elevated $[Ca^{2+}]_i$.

Interestingly, various levels of polyspermy induce equivalent increases in IP_3 [26]. Whether 1, 3, 10 or 75 sperm enter the egg, the IP_3 peak value is similar. That is, the binding of one sperm is

[1] See abbreviations.