Cellular and Molecular Mechanisms Leading to Cortical Reaction and Polyspermy Block in Mammalian Eggs

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ABSTRACT Following fusion of sperm and egg, the contents of cortical granules (CG), a kind of special organelle in the egg, release into the perivitelline space (cortical reaction), causing the zona pellucida to become refractory to sperm binding and penetration (zona reaction). Accumulating evidence demonstrates that mammalian cortical reaction is probably mediated by activation of the inositol phosphate (PIP_2) cascade. The sperm-egg fusion, mediated by GTP-binding protein (G-protein), may elicit the generation of two second messengers, inositol 1,4,5 triphosphate (IP_3) and diacylglycerol (DAG). The former induces Ca^{2+} release from intracellular stores and the latter activates protein kinase C (PKC), leading to CG exocytosis. Calmodulin-dependent kinase II (CaMKII) may act as a switch in the transduction of the calcium signal. The CG exudates cause zona sperm receptor modification and zona hardening, and thus block polyspermic penetration. Oolemma modification after sperm-egg fusion and formation of CG envelope following cortical reaction also contribute to polyspermy block. *Microsc. Res. Tech. 61:342–348, 2003.*

INTRODUCTION

Fertilization is generally considered a process of fusion between a haploid spermatozoon with an oocyte to create a diploid zygote. Physiological polyspermy, or penetration of the egg cytoplasm by more than a single spermatozoon, occurs in numerous species including insects, reptiles, and birds (Tarín, 2000), while polyspermy is considered an abnormal phenomenon in mammals, resulting in developmental failure of the zygote. Polyspermy in humans mostly results in spontaneous abortion, although births of tetraploid or triploid children have been reported (Dean et al., 1997; Roberts et al., 1996; Sherard et al., 1986; Shiono et al., 1988; Uchida and Freeman, 1985). These polyploidy births are characterized by severe malformations and multiple abnormalities (Doshi et al., 1983; Kjaer et al., 1997; Pitt et al., 1981). Recent studies in pig, a species with a high incidence of polyspermy under physiological conditions, suggest that this species may be an extraordinary case, since pig oocyte cytoplasm has the capability to remove the accessory sperm (Xia et al., 2001), and some poly-pronuclear pig eggs can develop to term if accessory sperm do not interrupt the embryo genome (Han et al., 1999). Polypronuclei can participate in karyosyngamy and the resulted polyploid eggs can develop to fetuses of diploid, triploid, or a mosaic with both diploid and tetraploid cells, but live piglets derived from transfer of polyploid eggs were diploid (Han et al., 1999). Under most circumstances in mammals, mating occurs before ovulation and a defense against polyspermic fertilization is established rapidly after fertilization. This block is both stable and long lasting (Hunter et al., 1998).

Following sperm penetration, cortical granules, a special organelle in eggs, released their contents into the perivitelline space (PVS) in an event that is termed the cortical reaction. CG exudates alter the properties of zona pellucida (ZP), which is known as zona reaction, and thus block polyspermic penetration. The incorporation of sperm membrane into the egg plasma membrane during gamete fusion also participates in polyspermy block at the level of oolemma. Numerous signal molecules have been shown to play important roles in cortical reaction and polyspermy block, and this article will review the current knowledge of related signal molecules/cascade involved in these processes.

MECHANISMS OF THE CORTICAL REACTION

In mammals, CGs reside in the cortex of a meiotic metaphase II (MII) stage oocyte. Sperm penetration or parthenogenetic activation of oocytes induces the exocytosis of CG contents known as cortical reaction, which brings about changes that prevent polyspermic penetration. Like in other secretory cells, CG exocytosis of oocytes involves a signal cascade.

Signal Molecules Involved in Cortical Reaction

Ca^{2+}. At fertilization, the release of intracellular Ca^{2+} is necessary and sufficient for most, if not all, of the major events of egg activation. One of the earliest events that is induced by Ca^{2+} rise is CG exocytosis (Abbott and Ducibella, 2001). Calcium ionophore A23187 stimulates CG exocytosis (Ducibella et al., 1993; Wang et al., 1997a), while the introduction of Ca^{2+} chelator BAPTA (1,2-bis(o-aminophenoxy)ethane-

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N,N,N′,N′-tetraacetic acid) into eggs inhibited Ca^{2+} transients and CG exocytosis induced by sperm (Kline and Kline, 1992). Recent studies show that the initial Ca^{2+} rise during rat egg activation is followed by segregation in the pathway. A relatively low Ca^{2+} rise is sufficient to induce a partial cortical reaction, while a higher level of Ca^{2+} is required to complete the cortical reaction (Raz et al., 1998a). Studies have suggested that CG exocytosis in mature eggs is dependent upon calcium-dependent proteins (Abbott and Ducibella, 2001).

IP₃. By using a function-blocking monoclonal antibody to the inositol 1,4,5 triphosphate (IP_3) receptor/Ca^{2+} release channel, Miyazaki et al. (1993) demonstrated that IP_3-induced Ca^{2+} release (IICR) from intracellular stores operates at fertilization of the hamster egg and that IICR is essential in the initiation, propagation, and oscillation of the sperm-induced Ca^{2+} rise. CG exocytosis is dependent primarily on an IP_3-mediated elevation of intracellular Ca^{2+} (Abbott et al., 1999). Microinjection of IP_3 into hamster and sheep oocytes induces CG release, and this effect is pH-dependent (Cran et al., 1988). Furthermore, microinjection of IP_3 into MII mouse eggs results in an extent of ZP2 conversion similar to that observed following fertilization (Ducibella et al., 1993), while microinjection of monoclonal antibody18A10, which binds to the IP_3 receptor and inhibits IP_3-induced Ca^{2+} release, inhibits in a concentration-dependent manner the ZP2 to ZP2f conversion (Xu et al., 1994).

Phospholipase C (PLC) mRNA and proteins are detected in mouse oocytes and PLC inhibitor exerts an inhibitory effects on oocytes activated by sperm. Thus, stimulation of PLC to generate IP_3 may play a critical role in egg activation (Dupont et al., 1996).

Calmodulin-Dependent Kinase II (CaMKII). CaMKII is inactive in MII arrested mouse oocytes. It is transiently activated in response to a rise in intracellular Ca^{2+} upon activation with ethanol (Winston and Maro, 1995). Ca^{2+}/CaMKII activation is the primary event leading to inactivation of both cytostatic factor (CSF) and maturation promoting factor (MPF) (Dupont, 1998). A role of CaMKII in promoting CG exocytosis has been revealed in sea urchins eggs by antibody injection (Steinhardt and Alderton, 1982). Whether CaMKII activation is involved in CG exocytosis is still not well defined. There is a report indicating that CaMKII activity is associated with second polar body emission and pronuclear formation but not with CG exocytosis when mouse eggs are activated by A23187 or 12-O-tetradecanoyl pborbol 13-acetate (TPA) (Inagaki et al., 1997), while other authors have recently proved that CaMKII activity increases during mouse oocyte maturation and it acts as a switch in the transduction of the calcium signal triggering mammalian egg activation including cortical reaction (Abbott et al., 2001; Tatone et al., 1999). When mouse eggs are incubated for 30 min in the presence of KN-93, an inhibitor of CaMKII, and induced to activate by ethanol, KN-93 elicits a marked reduction in CG exocytosis (Tatone et al., 1999).

Protein Kinase C. At fertilization, the spermatozoon causes the generation of two important second messengers in the egg, IP_3 and diacylglycerol, and the latter targets to PKC. It is well known that PKC participates in sperm acrosomal exocytosis (Breitbart et al., 1997), a process similar to CG exocytosis. Different PKC isoforms have recently been found to exist in rodent oocytes (Luria et al., 2000; Raz et al., 1998b). Physiological activation of MII eggs by sperm induces CG exocytosis associated with significant translocation of PKCα and PKCβ to the plasma membrane in the mouse (Luria et al., 2000). PKC activation by diacylglycerol (DAG) induced CG exocytosis (Ducibella et al., 1993; Sun et al., 1997). Phorbol esters, which mimic the action of DAG, also cause CG exocytosis (Jones, 1998) and ZP2-ZP2f conversion (Ducibella et al., 1993). PKC activation by phorbol ester also induces CG exocytosis in rat and pig oocytes (Raz et al., 1998a, c; Sun et al., 1997, 2001a). The induction of CG exocytosis by phorbol esters may bypass the Ca^{2+} rise (Sun et al., 1997; Raz et al., 1998a). Although numerous studies have shown that CG exocytosis is a PKC-dependent event, phorbol esters do not faithfully mimic CG release in fertilized eggs, resulting in an atypical pattern of CG release and, furthermore, PKC inhibition results in no detectable inhibition in treated fertilized eggs (Ducibella and LeFevre, 1997). A more recent study showed that PKCα,-βI, and γ were expressed in pig oocytes, while only PKCα and -βI were translocated to the plasma membrane after sperm penetration. Furthermore, eggs injected with PKCα isoform-specific antibody failed to undergo CG exocytosis PMA after treatment or fertilization. These results suggest that conventional PKCs, especially the α isoform, regulate CG exocytosis in pig eggs (Fan et al., 2002).

G-Proteins. A possible mechanism by which sperm activate CG exocytosis of oocytes is the G-protein-mediated signal-transducing pathway (Cran et al., 1988). In the sea urchin, the sperm activates G-protein, which in turn stimulates production of IP_3, leading to an elevation in free calcium and CG exocytosis (Turner et al., 1986). A GTP-binding protein activator A1F4 induces CG exocytosis in mouse eggs (Tahara et al., 1996).

Activation of G-protein with guanosine-5′-0-(3′-thiotriphosphate) (GTP-gamma-S), a hydrolysis-resistant analog of guanosine triphosphate (GTP), also causes changes normally occurring in fertilization including CG exocytosis in pig oocytes (Macháty et al., 1995). In addition, microinjection of mRNA encoding the rat M1 muscarinic receptor, a G protein-coupled acetylcholine (ACh) receptor, in combination with the addition of ACh in the medium leads to CG exocytosis in pig oocytes (Kim et al., 1998). Injection of GTP-gamma-S induces CG exocytosis in both hamster and sheep eggs (Cran et al., 1988). Williams et al. (1998) examined the role of Gq family of G proteins in both sperm-independent (muscarinic receptor-mediated) and sperm-induced egg activation using a function blocking antibody raised against the common C-terminal region of Gq and G11 proteins, and found that although activation of Gq family G proteins can result in egg activation events, it is unlikely that these proteins are used by sperm to initiate egg activation at fertilization in mouse.

Rabphilin-3A is a putative target protein for Rab3A, a member of the small G-protein superfamily. Rabphilin-3A has been shown to participate in Ca^{2+}-dependent CG exocytosis at fertilization in mouse eggs. Both mRNA and protein of Rabphilin-3A are detected in

mouse eggs. Furthermore, Rabphilin-3A protein is located in the cortical region in eggs. When NH2- or COOH-terminal fragment of recombinant Rabphilin-3A is injected into the mouse eggs, both inhibit CG exocytosis in a dose-dependent manner (Masumoto et al., 1996).

SNAP-25. Synaptosome-associated protein of 25 kD (SNAP-25) has been shown to play an important role in Ca^{2+}-dependent exocytosis in neurons and endocrine cells (Zhang et al., 2002). Both mRNA and protein exist in MII mouse eggs. After microinjection of an outline neurotoxin A (Bunt/A), which selectively cleaves SNAP-25, sperm-induced CG exocytosis is significantly inhibited, and the inhibition is attenuated by co-injection of SNAP-25, suggesting that SNAP-25 may be involved in Ca^{2+}-dependent CG exocytosis during fertilization in mouse eggs (Ikebuchi et al., 1998).

Role of Microfilaments in Cortical Granule Translocation and Exocytosis

During oocyte maturation, CGs were driven by microfilaments to move peripherally, assuming a position adjacent to the plasma membrane except for the area overlying the MII spindle (Longo, 1985; Sun et al., 2001a,b). An extensive microfilament network is also observed in mammalian oocyte cortex (DiMaggio et al., 1997; Kim et al., 1996; Sun et al., 2001a), but they are not required for the anchorage of CGs to the cortex (Connors et al., 1998; Sun et al., 2001a). Treatment of mouse or hamster eggs with cytochalasin B evidently blocked the release of CGs induced by sperm penetration, and microfilament stabilization by jasplakinolide also prevented CG exocytosis following artificial activation of mouse oocytes, suggesting that CG exocytosis requires microfilaments in these species (DiMaggio et al., 1997; Tahara et al., 1996; Terada et al., 2000). However, microfilaments are not involved in CG exocytosis in pig oocytes (Sun et al., 2001a).

Mechanisms Leading to the Development of Cortical Reaction Competence of Oocytes

In mammalian oocytes, CGs are still present in the penetrated oocytes at the GV stage and immature oocytes do not have the ability to block polyspermic penetration (Abbott et al., 2001; Ducibella, 1996; Wang et al., 1997b,c). The ability of oocytes to release CGs after sperm penetration develops after GVBD, and this ability is not fully developed until MII stage or near the time of ovulation (Abbott et al., 2001; Ducibella, 1996; Wang et al., 1997b). The incompetence of fertilized immature oocytes to undergo CG exocytosis could be due to an insufficient number of CGs, their distance from the plasma membrane, but studies indicated that neither accounts for the incompetence of GV mouse oocytes to undergo cortical reaction (Ducibella, 1996). A PKC-sensitivity develops prior to meiotic maturation, since phorbol ester, 12-O-tetradecanoyl phorbol 13-acetate (TPA), stimulates both CG exocytosis and ZP2 conversion in GV mouse oocytes (Ducibella et al., 1993).

As mentioned above, CG release in mature oocytes is dependent upon the Ca^{2+} rise and calcium-dependent proteins, especially protein kinases. The incompetence of preovulatory oocytes to release CGs likely is not due to a lack of proximity of CGs to the egg's primary calcium store, the endoplasmic reticulum (Abbott et al., 2001), but probably due to deficiencies in the ability to release and respond increases in intracellular Ca^{2+} (Abbott and Ducibella, 2001). GV stage mouse or hamster oocytes do not elevate Ca^{2+} to the same extent as in mature oocytes after fertilization or IP_3 injection (Fujiwara et al., 1993; Mehlmann and Kline, 1994). Microinjection of IP_3 into mouse MII eggs resulted in an extent of ZP_2 conversion similar to that observed following fertilization, whereas little conversion occurred in GV-intact oocytes similarly injected (Ducibella et al., 1993). These results suggest that the IP_3-induced Ca^{2+} release mechanism is deficient in GV-stage oocytes and such a mechanism develops during oocyte maturation after maturation has resumed (Ducibella et al., 1993; Fujiwara et al., 1993; Mehlmann and Kline, 1994).

When sperm factor was microinjected into mouse oocytes, 86% CGs released in MII oocytes, while only less than 1% CGs released in GV oocytes, although Ca^{2+} oscillations were induced. Thimerosal or electrical pulses induce Ca^{2+} oscillations in both MII and GV oocytes, but a significantly larger proportion of CGs released in MII oocytes than in GV oocytes after treatment (Abbott et al., 1999). These results suggest that mechanisms downstream of Ca^{2+} rise are developmentally established during the acquisition of the CG exocytosis ability of oocytes.

A recent study found that CaMKII expression increased 150% and its activity increased 110% during mouse oocyte maturation, suggesting that maturation-associated increase in CaMKII correlates with the acquisition of secretory competence of oocytes (Abbott et al., 2001).

Cortical Reaction Induced by Injected Sperm or Sperm Fractions

The cortical reaction in mammalian oocytes is induced following sperm-egg membrane fusion. However, the physiological cascade of gamete interaction events is bypassed during intracytoplasmic sperm injection (ICSI). In ICSI, as in physiological fertilization, oocyte activation is a prerequisite for CG exocytosis (Chetler et al., 1998). Injections of sperm preparations into mammalian oocytes have been shown to elicit persistent Ca^{2+} oscillations, which, in turn, initiate all events including CG exocytosis (Maleszewski et al., 1996; Stice and Robl, 1990; Wu et al., 1998). In the pig oocytes, a crude extract isolated from boar sperm induced an immediate rise in free Ca^{2+} concentration, followed by repetitive Ca^{2+} transients and CG exocytosis (Macháty et al., 2000). Such an effect was totally abolished by heat or trypsin treatment of the extract (Macháty et al., 2000). However, a lag period before Ca^{2+} oscillations exists when a spermatozoon is injected into the ooplasm (Ducibella, 1996), and CG exocytosis is delayed. For example, at 10 h after sperm injection, when a single pronucleus was formed, abundant CGs still existed in the subplasmalemmal region in equine oocytes; all CGs were released at 20 hours when 2 pronuclei formed (Grondahl et al., 1997). Truncated c-kit is specially located in the residual sperm cytoplasm. Microinjection of recombinant truncated c-kit into MII-arrested mouse oocytes causes complete

oocyte activation including CG exocytosis (Sette et al., 1997).

MECHANISMS FOR POLYSPERMY PREVENTION

The fusion of sperm and egg and the release of CG contents after cortical reaction leads to changes in the zona pellucida, oolemma, and privitelline space for polyspermy block. Depending on the species, polyspermic block resides either at the zona pellucida, or the egg plasma membrane, or both. For example, polyspermy is primarily blocked by zona changes in hamster, goat, ovine, and bovine oocytes, by oolemma changes in rabbit oocytes, and by both in mouse, rat, guinea pig, and cat oocytes (Yanagimachi, 1994).

Zona Reaction

In most mammals studied to date, the primary block to polyspermy occurs at the zona pellucida, the extracellular coat surrounding the egg, after CG exocytosis. The CG exudates act on the zona pellucida, causing biochemical and structural changes that make zona pellucida lose its ability to bind sperm and to be penetrated by sperm previously bound to the zona pellucida.

Mammalian oocyte zona pellucida consists of three glycoproteins, ZP1, ZP2, and ZP3. ZP2 and ZP3 are present as heterodimeters and act as the primary and secondary sperm receptors, respectively, in mouse oocytes. Acrosome-intact sperm bind to the O-linked oligosaccharides that are covalently linked to ZP3 and undergo acrosome reaction. Then the acrosome-reacted sperm bind by their inner acrosomal membrane to ZP2. Sperm receptors ZP3 and ZP2 are modified to become ZP2f and ZP3f by CG exudates shortly after fertilization and thus polyspermy is prevented (McLeskey et al., 1998; Wassamman, 1994). An uneven distribution of different sugar residues in the rat ZP and postfertilization changes in the distribution of β-galactose are supposed to be correlated with polyspermy block (Raz et al., 1996). Identified CG contents that may be involved in zona reaction are described as follows.

Proteinases. Proteinases, including tissue-type plasminogen activator (tPA), are released from mammalian oocytes following fertilization and artificial activation. These proteinases modify the ZP so that it is no longer receptive to sperm (Warssarman, 1994). The cortical granule proteinase catalyzes the proteolysis of ZP2 and causes the decrease in solubility of the ZP (hardening) (Moller and Warssarman, 1989). A trypsin-like proteinase is released from the cortical granules and modifies the zona to block polyspermy (Barros and Yanagimachi, 1971; Gwatkin et al., 1973; Wolf and Hamada, 1977). tPA may play an important role in zona reaction. Sperm penetration or A23187 treatment induced rat oocytes to release tPA into the surrounding medium and caused ZP hardening. The presence of monoclonal tPA antibody during in vitro activation of oocytes by A23187 inhibited zona hardening and further sperm penetration, suggesting a possible role of tPA release during cortical reaction in zona reaction and polyspermy block (Zhang et al., 1992). Another trypsin inhibitor-insensitive proteinase was separated in mouse oocytes and it modified ZP2 to produce ZP2f, and caused zona hardening (Moller and Wassaman, 1992). In pig, a 90-kD protein is degraded by protease released from the oocyte at fertilization, thereby leading to the block of polyspermy (Hatanaka et al., 1992).

Ovoperoxidase. Ovoperoxidase is one of several oocyte-specific proteins that are stored within sea urchin CGs and it is incorporated in the newly formed fertilization envelope after cortical reaction and thus participates in polyspermy block (LaFleur et al., 1998). Mouse cortical granules also contain an ovoperoxidase as revealed by cytochemical techniques and it hardened ZP after ionophore treatment (Gulyas and Schmell, 1980; Schmell and Gulyas, 1980).

N-Acetylglucosaminidase. N-acetylglucosaminidase is localized in the CGs of mouse oocytes by immunoelectronic microscopy (Miller et al., 1993). During gamete interaction at fertilization, sperm surface beta-1,4-galactosyltransferase (GalTase) binds to terminal N-acetylglucosamine residues on specific oligosaccharides of the zona glycoprotein ZP3. N-acetylglucosaminidase was released at fertilization and inactivated the sperm GalTase-binding site of the ZP, and thus blocked sperm binding. Inhibition of N-acetylglucosaminidase released from activated eggs, with either competitive inhibitors or with specific antibodies, resulted in polyspermic binding to the zona pellucida (Miller et al., 1993).

Others. A 60-kD protein, calreticulin (CRT), is identified to exist in the CGs of hamster eggs. CRT is exocytosed during cortical reaction in response to egg activation and it may be involved in polyspermy prevention (Munoz-Gotera et al., 2001). A 32-kD protein identified by an antibody raised against mouse CG contents may also be involved in the block to polyspermy (Gross et al., 2000). In addition, a 75-kD glycoprotein known as p75 (Pierce et al., 1990, 1992), a heparin-binding placental protein (Sinosich et al., 1990a, b), and numerous glycoconjugates (Hoodbhoy and Talbot, 2001) were localized in mammalian CGs and they are released from oocytes upon activation. The functions of these components on the zona reaction are still not known.

Plasma Membrane Block

The egg plasma membrane block to polyspermy has been observed in several species including humans (Horvath et al., 1993; Sengoku et al., 1999; Wolf, 1981). The nature of the plasma membrane block response differs somewhat between species. In the rabbit, the plasma membrane block represents the primary block to polyspermy, in that the zona reaction is not observed (Overstreet and Bedford, 1974; Wolf, 1981), while in most species, both zona block and oolemma block function. In some lower species, a depolarization of the plasma membrane potential, called the fertilization potential, prevents further spermatozoa from fusing with the oocytes immediately after sperm penetration. However, such an electrical polyspermy block at the level of the plasma membrane is not observed in mammals (Tarín, 2000). The accumulation of CG contents on oolemma and the fusion of CG membrane with oolemma could play roles in the establishment of an oolemma block to polyspermy. However, accumulating evidence suggests that the contents of the egg's cortical granules do not play a role in the establishment of the plasmalemma block to polyspermy, while sperm membrane incorporation into the oolemma contributes to

Dthe changes in the oolemma that block polyspermy. Reinsemination of ICSI mouse oocytes showed that ICSI oocytes did not develop an oolemma block to sperm penetration, although CG exocytosis had occurred (Maleszewski et al., 1996). Similarly, activation of mouse eggs with either ethanol or strontium chloride caused CG exocytosis, but did not result in a block to polyspermy (Horvath et al., 1993). The same conclusion is obtained in human eggs. Pronuclear human oocytes fertilized by sperm were not penetrated after re-insemination, while a majority of zona-free human oocytes fertilized by intracytoplasmic sperm injection or activated by parthenogenetic activation were penetrated (Sengoku et al., 1999).

Formation of Cortical Granule Envelope

After fertilization of mouse, hamster, and human oocytes, CG exudates accumulate in the PVS, forming a new coat, termed the CG envelope, around the fertilized eggs. This envelope is possibly related to blocking polyspermic penetration at the level of PVS or the oolemma by hindering gamete interaction or modifying an incoming sperm (Dandekar and Talbot, 1992). In addition to numerous glycoproteins such as Canavalia ensiformis agglutinin (ConA) and Lens culinaris agglutinin (LCA) in the CG envelope (Hoodbhoy and Talbot, 1994, 2001), two CG-derived polypeptides, p62 and p56, were found to exist in this envelope of hamster eggs (Hoodbhoy et al., 2000, 2001). Formation of a CG envelope following the cortical reaction was also reported in marsupial oocytes (Dandekar et al., 1995).

Others

In addition to the oocyte's ability to block polyspermy, female reproductive tract limits the arrival of sperm to the fertilization site. For example, of the 50 million or so mouse sperm that begin the journey, only 100–200 sperm actually reach the site of ovulated eggs (Wassarman, 1994). In the pig, the polyspermy rate is significantly higher for in vitro matured oocytes than for ovulated oocytes in an in vitro fertilization system (Wang et al., 1998). When an oviduct-specific glycoprotein (pOSP) was added before or during in vitro fertilization, sperm binding to the zona pellucida, penetration and polyspermy were reduced, and this effect was blocked with a specific antibody to pOSP (Kouba et al., 2000). During in vitro fertilization of bovine oocytes, the cumulus cells engulfed numerous sperm, which may contribute to the prevention of polyspermic fertilization (Sun, 2000).

CONCLUDING REMARKS

Mammalian oocytes develop their ability to undergo cortical reaction during maturation. Cortical reaction of oocytes is probably mediated by activation of the inositol phosphate (PIP_2) cascade. The sperm-egg fusion, mediated by G-protein, may elicit the generation of two important second messengers, IP_3 and diacylglycerol (DAG). The former induces Ca^{2+} release from intracellular stores and the latter activates PKC, thus leading to the membrane fusion of the oocyte and CGs (Fig. 1). During this process, CaMKII may act as a switch in the transduction of the calcium signal. Injected sperm or sperm factors (extract) may also induce cortical reaction through inducing Ca^{2+} rise. Mechanism of IP_3-induced calcium and mechanisms downstream of Ca^{2+} rise such as the maturation-associated increase in CaMII activity develop during oocyte maturation.

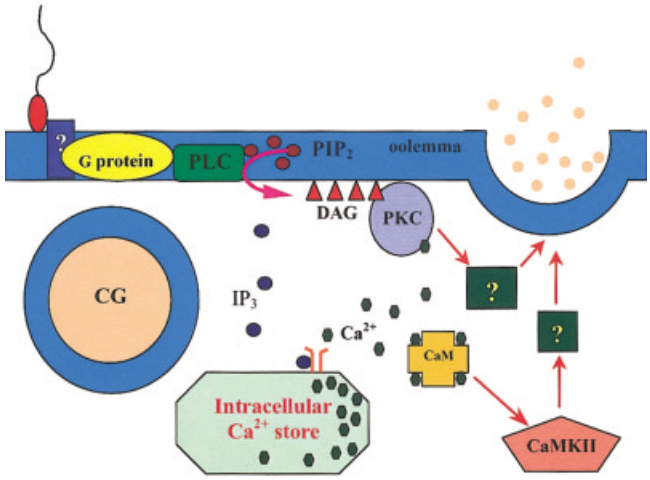

Fig. 1. Possible mechanism of CG release induced by spermatozoon. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Sperm-egg fusion and cortical reaction lead to changes that block polyspermy. The block to polyspermy involves zona reaction, oolemma modification, and CG envelope formation. The proteinases, ovoperoxidase, and N-acetylglucosaminidase, are though to bring about changes in the zona pellucida (sperm receptor modification and zona hardening). Sperm membrane incorporation into oolemma contributes to the changes in the oolemma that block polyspermy. CG envelope formation is possibly related to blocking polyspermic penetration at the PVS level. Other mechanisms unrelated to gamete fusion and egg cortical reaction limit the number of sperm at the fertilization site or sperm-egg interaction and thus play an additional role in polyspermy block.

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