REVIEW ARTICLE

Roles of protein kinase C in oocyte meiotic maturation and fertilization*

FAN Hengyu, TONG Chao, CHEN Dayuan and SUN Qingyuan**

(State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, China)

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Abstract Protein kinase C (PKC) is a superfamily of Ser/Thr protein kinases that is distributed widely in eukaryotes. It plays key regulatory roles at multiple steps of oocyte meiotic maturation and fertilization. During the process of meiotic maturation, the activation of PKC in cumulus cells stimulates meiotic maturation, whereas the activation of PKC in oocytes results in the inhibition of germinal vesicle breakdown. PKC activity increases following the meiotic maturation, and decreases at the transition of metaphase/anaphase in meiosis I, so as to facilitate the release of the first polar body and the entry of meiosis II. In fertilization of mammalian oocytes, PKC may act as one of the downstream targets of Ca^{2+} to stimulate the cortical granule exocytosis, release the oocytes from MII arrest and to induce pronucleus formation. PKC is also involved in the regulation of maturation promoting factor (MPF) and mitogen-activated protein kinase (MAPK). Several PKC isoforms have been identified in mammalian oocytes, and there is evidence showing that classical PKCs may be the principal mediator of oocyte cortical reaction.

Keywords: protein kinase C, oocyte, meiosis, fertilization.

The oocytes in the adult vertebrate ovary are arrested at the diplotene of the first meiotic prophase. During every estrus cycle, with the stimulation of the pituitary gonadotrophin surge, the oocytes reinitiate meiosis I and progress to the metaphase of the second meiotic division (MII), at which stage their cell cycle arrests again. In most cases in vertebrate, ovulated oocytes stay in MII stage until fertilization or parthenogenetic activation. Usually, the period from meiosis reinitiation to MII stage is termed oocyte maturation, while the oocyte development after the release of MII arrest is called egg activation. The difference between the first and the second meioses is that in the former case cell transits from interphase to metaphase, while in the latter case cell transits from metaphase to interphase.

The combination process of male and female germ cells to form a zygote is called fertilization, which is the start point of a life. The oocyte maturation, fertilization and activation are not only the core points of animal reproduction, but also an ideal research model of cell cycle regulation and signal transduction. The development in stem cell technology and animal cloning needs deeper understanding of the molecular mechanism in oocyte development and fertilization. Protein kinase C (PKC), which was found in the 1980s, is a pivotal regulatory molecule in the cell and plays important roles in several processes during oocyte maturation and fertilization. In the last five years, our laboratory has used the oocytes of mice, rat, pig and rabbit as materials to probe the roles of PKC during oocyte maturation and fertilization. The present paper reviews the research progresses in this area.

1 Biochemical description of PKC

PKC, a family of serine/ threonine protein kinases, exists widely in eukaryotic cells. It comprises 12 isoforms and can be divided into three major groups: classical PKC (cPKC α, βI, βII and γ), novel PKC (nPKC δ, ε, θ, η and μ), and atypical PKC (aPKC λ/τ and ζ). The activation of cPKCs depends on calcium and diacyglycerol (DAG), while the activation of nPKCs is calcium-independent but is still DAG-dependent[1]. aPKCs are regulated by neither calcium nor DAG, but by some types of lipid second messenger. Recently, some proteins were found to

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** To whom correspondence should be addressed. E-mail: sunqy@panda.ioz.ac.cn or sunqy1@yahoo.com

bind specifically with the regulatory domain of aPKC and regulate the activity of aPKCs[2]. PKC βI and βII are encoded by the same gene but formed by different splicing[3]. All the isoforms of PKC have one chain of peptide whose molecular weight is about 70 to 90 kD. PKC is composed of an N-terminal regulatory domain (about 20 ~ 40 kD) and a C terminal catalyzing domain (about 45 kD). According to the existence of conservatory amino acids, PKC is divided into 4 conserved domains (C1 ~ C4, nPKC and aPKC lacking C1) and 5 variable domains (V1 ~ V5). C1 domain is DAG and phobolester binding domain and contains a pseudo-substrate sequence which can auto-inhibit PKC activity. The C2 domain of some PKC isoforms contains calcium-binding sites. The C3 and C4 domains form the catalyzing center which include ATP and substrate binding domains. Usually, the pseudo-substrate binds its own catalyzing domain, which makes PKC inactivate until PKC activator pulls the pseudo-substrate out of the catalyzing center. The inactive is PKC usually distributed in cytoplasm and translocates to the plasma membrane after being activated[4].

2 Roles of PKC in meiotic maturation of oocytes

2.1 PKC and the initiation of meiosis

Fully grown oocytes liberated from their follicles spontaneously reinitiate meiosis I *in vitro*, characterized by germinal vesicle breakdown (GVBD), chromatin condensation, spindle formation, emission of the first polar body, and progression to metaphase of the second meiotic division (MII).

Several studies on the roles of PKC in oocyte maturation have been done in many species; however, the results did not consist with each other. Some research found that PKC inhibits the meiotic resumption of oocytes[5,6], while the other results show that PKC stimulates this process[7,8]. This contradiction can be explained by the compartmentation of PKC in cumulus-oocyte complex (COC). If the PKCs in cumulus cells are activated, the meiotic resumption will be triggered. But if the PKCs in oocytes are activated, the meiosis resumption will be inhibited. In denuded oocyte (the oocyte without cumulus cell), phorbol 12-myristate 13-acetate (PMA), a PKC activator, strongly inhibits GVBD, while the PKC inhibitor calphostin C can overcome the inhibitory effects of PMA on GVBD. According to our result, treating the denuded oocytes with 1.62 nmol/L PMA for 5 min could inhibit GVBD in more than 90% of the oocytes. The other PKC activators have the same results[5,6]. But Down's results[7] suggest that PMA and other PKC activators promoted GVBD in cumulus-enclosed oocytes. Furthermore, PKC activators stimulated cumulus cell expansion. The different effect of PKC activation in cumulus cells and oocytes on oocyte development maybe due to the different isoform expression in these two cell types. The different PKC isoforms activate different down stream molecules and exert different effect. PKC α, β, δ and ζ can be found in both cumulus cells and oocytes, but PKC ε is expressed specifically in cumulus cells[7]. The further work should be done to find whether different isoforms have different down stream signal transduction in COCs. PKC activation may stimulate the synthesis of some key molecules in cumulus cells to facilitate oocyte meiosis, which can be transported through the gap junction and get into oocyte. The other possibility is that PKC may induce oocytes to produce some secretary factor, which can stimulate GVBD by paracrine pathway. Although these results suggest that PKC can regulate oocyte maturation, it may not participate in the gonadotrophin-induced meiosis resumption under physical conditions. PKC may be involved in a substitute pathway that promotes meiosis maturation induced by the non-gonadotrophin ligand, such as epidermal growth factor (EGF).

The mechanism of how PKC inhibits oocyte maturation is unclear now. Mitogen-activated protein kinase (MAPK) is a pivotal protein kinase that regulates the meiosis. It is phosphorylated during oocyte maturation[9,10]. We found in mouse[5], rat[6] and porcine oocytes that PKC activator could inhibit GVBD and MAPK activation at the same time. PKC may inhibit an upstream molecule of MAPK pathway necessary for MAPK activation, and then inhibit GVBD. The role of PKC in oocyte maturation is shown in Fig. 1.

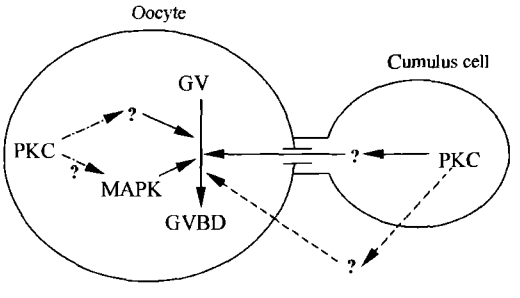

Fig. 1. Possible roles of PKC in the meiotic maturation of oocytes. "→" represents stimulation, and "⊣" represents inhibition.

Presently, the expression of cPKC α, βI, βII[11], nPKC δ[12] and aPKC λ[13] have been detected in mouse oocytes at GV stage. In GV oocytes, cPKC α, βI and βII are distributed evenly in cytoplasm and cannot be detected in germinal vesicle. PKC α tranlocates to membrane after phorbol ester treatment, while there is no obvious change of the distribution of cPKC βI and βII. Since cPKC α is the only kinase activated by the TPA treatment, it may be responsible for GVBD inhibition[11]. We have detected the expression of three cPKC isoforms in porcine oocytes by Western blotting. The quantity of expression remains stable during GV and MII stage, while their subcellular distribution changes with the oocyte development. They concentrate to the nucleus at GV stage, but translocate to cytoplasm after GVBD[14]. These PKC isoforms may be involved in the regulation of chromatin condensation and nuclear membrane organization during oocyte maturation.

2.2 PKC and the metaphase/anaphase transition of meiosis I

Since PKC participates in the regulation of meiosis entrance and exit, it may also have some functions in MI/MII transition. Activation of PKC after GVBD in mouse oocytes inhibits the development of oocytes to MI stage[1)]. The activity of PKC rises with the development of oocytes and becomes the highest at the late MI stage. Inhibition of PKC activity reduces MPF activity quickly and then induces the MI/MII transition. However, the activity of MAPK is not affected by the PKC inhibition. PKC δ is concentrated on the MI spindle and then around the chromosome in MII oocytes. An abnormal long MI stage was found in LTXBO strain of mouse. In these oocytes, PKC maintained a high level of activity during MI stage. If PKC activity was reduced artificially, the oocytes could exit MI stage[12]. In our experiments, PKC activator PMA induced MI arrest in porcine oocytes that had already undergone GVBD. This result suggests that the decrease of PKC activity is necessary for porcine oocyte MI/MII transition[15].

It has been known that the regulatory factors for maintaining MI and MII arrest are different. MOS, an important component of cytostatic factor (CSF), is a key factor for the maintenance of MII arrest, but it is not necessary for MI arrest maintaining. Although mos knockout mouse oocyte cannot maintain MII arrest, the MI stage development is normal[16]. It is not fully known which factor regulates the activity of CSF at MI stage. However, the above research suggests that PKC may exert positive regulation to CSF during the MI stage.

3 Roles of PKCs in egg activation

During fertilization, sperms activate oocytes by binding the receptors on the surface of oocyte or releasing a dissolvable sperm factor to oocyte cytoplasm. The activation of oocytes including not only the early events such as cortical granule exocytosis and polyspermy blocking, but also later events, such as meiosis resumption and mitosis initiation. The interaction of sperm and oocyte induces the hydrolysis of phosphatidylinositol (PIP2) in oocyte plasma membrane to IP3 and DAG. IP3 induces the release of free calcium ion from calcium store in cell, while DAG activates PKC. The first event ever known after sperm and oocyte binding was the rising and periodical fluctuation of calcium concentration in cell, named calcium oscillation. Many research results suggest that calcium mediates cortical reaction and meiosis resumption in fertilized eggs. However, the target of calcium is not clear until now. PKC seems a likely candidate.

3.1 PKC and cortical reaction

In most animal oocytes, the sticky cytoplasm under the membrane with a few cell organs is called cortex. The cortical granules (CG), a group of membrane-bound secretary granules with diameter from 0.8 to 2 μm and composing specialized enzymes and glycoproteins, are located mostly in the cortex of unpenetrated mature oocytes. With the trigger of some physical or chemical stimulation, the calcium concentration in cytoplasm is increased. Calcium promotes the fusion of CG with oocyte membrane, which releases the enzymes that hydrolyze ZP2 glycoprotein and makes ZP3 lose the abilities of binding with sperm and induces capacitation. So the sperms penetrated lately cannot fuse with oocyte membrane. The whole process is named cortical reaction[17]. Cortical reaction is an important mechanism for oocytes to block polyspermy. Although it is not very clear how calcium induces CG excytosis, a lot of evidence suggests that PKC activation may mediate this process.

In our experiments, PKC activator 1-oleyl-2-

1) Our unpublished data.

acetyl-sn-glycerol (OAG) and PMA stimulated the CG exocytosis in porcine oocytes. Furthermore, CG exocytosis induced by PKC activator was blocked by staurosporine, a PKC inhibitor. It seemed that PKC activation is necessary for CG exocytosis in porcine oocytes[18]. The previous work showed that CG exocytosis induced by PKC activator was independent of the calcium concentration. Even using excessive calcium chelator BAPTA-AM to decrease the intracytoplasmic or intercytoplasmic calcium, PMA still can induce CG exocytosis. These results suggest PKC is indeed the downstream molecule of calcium. While, some researchers indicated that CG exocytosis induced by PKC was different from that induced by fertilization. For example, phorbol ester-induced CG exocytosis mainly happened on the equator of oocytes, while under the physical condition, CG exocytosis happened evenly on the whole oocyte. The other evidence showed that although PKC inhibitor inhibited phorbol ester-induced CG exocytosis, it could not block CG exocytosis induced by fertilization[19]. As a result, researchers began to probe into the roles of different PKC isoforms in cortical reaction induced by fertilization.

cPKC α, βI and βII can be detected in cytoplasm of MII mice[11] or rat[20] oocytes. After fertilization or TPA treatment, with CG exocytosis, cPKC α and βI translocated to membrane, which suggests that cPKC α and βI rather than cPKC βII may participate in CG exocytosis and egg activation.

In our experiment, with CG exocytosis, PKC isoforms migrated to plasma membrane to different extents after fertilization or parthenogenetic activation. The migration of PKC α was significant, while the translocalization of PKC βI and γ was not distinct. The further work showed that CG exocytosis induced by fertilization or parthenogenetic activation was completely inhibited by PKC α but not by PKC βI and γ antibody microinjection. These results suggest that the activity of PKC α is necessary for CG exocytosis in porcine oocyte. However, after *in vitro* fertilization, CG exocytosis in oocytes injected with PKC α antibody was not blocked completely. Cortical reaction could still happen in some oocytes. Furthermore, CG exocytosis happened in most eggs at about 20 hours after fertilization. These results suggest there is another substitute pathway regulating CG exocytosis, and the inhibition of PKC α cannot block cortical reaction completely[14].

Although we have clarified which PKC isoform participates in cortical reaction, there are still many questions to be answered. It is not clear which PKC downstream molecules mediate fertilization-induced cortical reaction. PKC may phosphorylate other kinases, cytoskeleton proteins, or membrane proteins in CG. It has been found that CaMKII and small G protein were pivotal molecules for cortical reaction, but whether they work as cooperators of PKC or function independently is still unknown[1)].

3.2 PKC and the completion of meiosis

There are still many incompatible reports about the roles of PKC in cell cycle regulation after fertilization. Some research showed that PKC activator such as PMA and OAG induced parthenogenetic activation in mice and rat eggs. But other results from mouse, rat and hamster showed that PKC was not the pivotal molecule in egg activation. Our results suggest that whether the mouse eggs can be activated depends on the age of the eggs. After emission of the first polar body, with the increase of the egg age, the sensitivity of eggs to PKC activators increases too. In other words, the older the egg is, the easier the PN formation is. Furthermore, the roles of PKC in egg activation have strong species specificity. In different species, even different strains, the response of eggs to PKC activator is different. For example, PMA cannot induce porcine egg activation[14,18]. We found that the Kunming mouse often used by Chinese researchers is not sensitive to PKC activators.

In mammalian eggs, the rise of calcium concentration may activate PKC and induce the break through of MII arrest. Some evidence supports the above point. First, the oocytes of mouse, rat or hamster treated with different types of PKC activator can induce oocyte to transform into interphase without the change of calcium concentration. Second, different PKC inhibitors can inhibit parthenogenetic activation induced by different stimulations. Colonna et al. found that when the mouse eggs were treated with PKC activator OAG, the activity of MPF was reduced quickly without the change of calcium concentration and the eggs enter anaphase. Usually, calcium ionophore A23187 induces MPF degradation and egg activation by raising the calcium concentration in oocyte. If PKC activity was inhibited, A23187 still

1) Sun, Q. Y. Cellular and molecular mechanisms leading to cortical reaction and polyspermy block in mammalian eggs. To be published.

induced calcium concentration to rise, but MPF didn't degrade, nor oocyte activation[21]. These results suggest that PKC activation is necessary to MII arrest break though and MPF degradation induced by calcium concentration rising is PKC activity dependent. Presently, it is not clear how calcium activates PKC. One possibility is calcium activates phospholipase C (PLC), which induces the formation of DAG which activates PKC. The other possibility is the calmodulin-dependent protein kinase II (CaMKII) may participate in the PKC activation induced by calcium. A lot of evidence showed that CaMKII is the direct target of calcium in mouse oocytes[22].

The mechanism of MPF regulation by PKC is different between mouse and amphibian. Although PKC is involved in array events in *Xenopus* oocytes activation, it does not participate in MPF degradation[23]. It is still a big question whether PKC activation is necessary to oocyte activation under physical conditions.

Our result showed that there is a cross-talk between PKC and MAPK during oocyte maturation and activation. It has been well known that MAPK is a key component of CSF. PKC activation induced MAPK inactivation and pronucleus formation in mouse[5,24] and rat[25] eggs. PKC activators PMA and diC8 induced MAPK inactivation and PN formation in a dose-dependent manner. Furthermore, the effect of PKC activator could be reversed by PKC inhibitors calphostin C and staurosporine. During the normal fertilization process, the PKC inhibitor mentioned above blocked MAPK inactivation and PN formation induced by sperm. These results suggest that the combination of egg and sperm may activate PKC and overcome MII arrest. Then PKC induced MAPK inactivation and PN formation by some unknown mechanisms. The role of PKC in oocyte activation is shown in Fig. 2.

How can PKC induce MAPK inactivation? It has been known that serine/threonine protein phosphorase-2A (PP2A) induces the inactivation of MAPK and its upstream kinase MEK. The expression of PP-2A has been detected in mouse oocytes[26]. We found that protein phosphatase is involved in MAPK activity regulation. When the activity of protein phosphatase was inhibited, the activity of MAPK increased steeply in porcine oocytes[27]. PKC-induced MAPK inactivation and PN formation could be inhibited by OA in mouse[5] and rat[6] oocytes. These results suggest that PKC may induce MAPK dephosphorylation and PN formation by activating PP-2A.

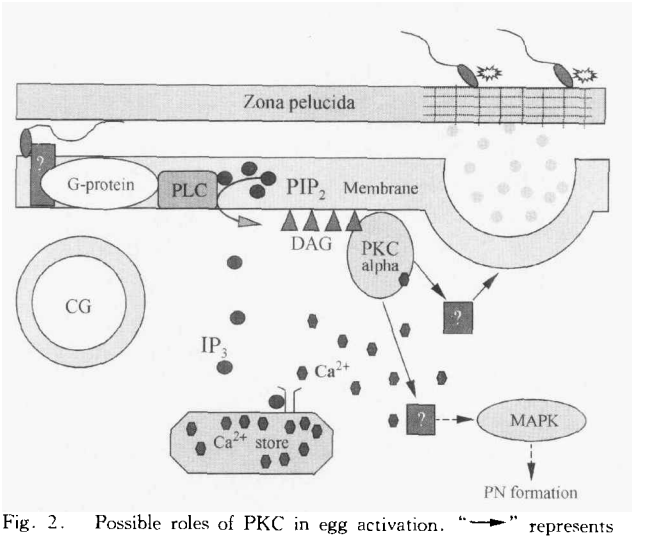

Fig. 2. Possible roles of PKC in egg activation. "→" represents stimulation, and "⊣" represents inhibition.

4 The comparison of roles of PKC in the first and second meiosis

There are some similarities between the two meiosis arrests: first, PKC activation always maintains or induces the completeness of pronuclear membrane. In GV oocytes, PKC inhibits GVBD, while it induces PN formation in MII eggs. Second, PKC always down regulates the activity of MAPK no matter which stage the oocytes are in. PKC inhibits the activation of MAPK before it is activated in GV oocytes and induces MAPK inactivation when it has been activated in MII eggs. Since induction of MAPK inactivation in MII eggs needs the higher concentration of PMA than inhibiting MAPK activation in GV oocytes, we think the former process is more difficult than the latter. Although we cannot predict whether similar mechanism is used by PKC between two meioses, we have found that the activity of serine / threonine protein phosphatase is indispensable. Because PKC neither inhibits GVBD in GV oocytes nor induces MII oocyte activation when the oocytes were treated with OA[5,6].

Fertilization is a process that needs the interaction of oocyte and sperm. The present review only summarizes possible roles of PKC in oocytes. Vast references showed that PKC also regulates the capacitation and acrosome reaction during fertilization.

5 Conclusion

As a core factor in cell signal transduction, PKC

is involved in oocytes meiotic maturation and fertilization. The research in PKC function has benefited from the using of many types of activators and inhibitors. With the deepening of research in this area, it is necessary to clarify the function of different PKC isoforms. While, the specific activators and inhibitors to different PKC isoforms obtained are limited, which makes the research in the function of single PKC isoforms limited. Furthermore, the study on PKC isoform function has only been focused on a few classical PKC isoforms, the further work should be done in the nPKC and aPKC isoforms. Presently, little is known about which molecule is the direct target of PKC in meiosis and fertilization.

In the last thirty years, calcium as the secondary messenger in eukaryotic cells attracts more and more attention. But as a simple cation, calcium is only a messenger and cannot be a function executer. It has been known that PKC and CaMK are the most important downstream molecules of calcium signal. They mediate several calcium functions by phosphorylating other proteins. PKC mediated calcium functions during fertilization include cell cycle regulation, nuclear membrane and spindle organization, and exocytosis as well.

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