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Minireview

Involvement of Mitogen-Activated Protein Kinase Cascade During Oocyte Maturation and Fertilization in Mammals[1]

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ABSTRACT

Mitogen-activated protein kinase (MAPK) is a family of Ser/Thr protein kinases that are widely distributed in eukaryotic cells. Studies in the last decade revealed that MAPK cascade plays pivotal roles in regulating the meiotic cell cycle progression of oocytes. In mammalian species, activation of MAPK in cumulus cells is necessary for gonadotropin-induced meiotic resumption of oocytes, while MAPK activation is not required for spontaneous meiotic resumption. After germinal vesicle breakdown (GVBD), MAPK is involved in the regulation of microtubule organization and meiotic spindle assembly. The activation of this kinase is essential for the maintenance of metaphase II arrest, while its inactivation is a prerequisite for pronuclear formation after fertilization or parthenogenetic activation. MAPK cascade interacts extensively with other protein kinases such as maturation-promoting factor, protein kinase A, protein kinase C, and calmodulin-dependent protein kinase II, as well as with protein phosphatases in oocyte meiotic cell cycle regulation. The cross talk between MAPK cascade and other protein kinases is discussed. The review also addresses unsolved problems and discusses future directions.

fertilization, gamete biology, kinases, meiosis, signal transduction

INTRODUCTION

Mammalian oocytes are arrested within ovarian follicles at the diplotene stage of the first meiotic prophase, which is also termed the germinal vesicle (GV) stage. Following stimulation by the pituitary LH surge in each estrus cycle, the fully grown oocytes reinitiate meiosis. The resumption of meiotic maturation is manifested by germinal vesicle breakdown (GVBD), followed by chromatin condensation and microtubule reorganization. These events lead to the formation of the metaphase spindle and subsequent completion of the first meiotic division, after which oocytes enter meiosis II and become arrested at metaphase II (MII) until fertilization or parthenogenetic activation. The oocytes released from the MII arrest complete the second meiotic division, undergo pronuclear formation, and subsequently enter the mitotic cell cycle.

It has long been of interest how the meiotic cell cycle of oocytes is regulated differently from the mitotic cell cycle of somatic cells and of early blastomeres. It has been well recognized that protein phosphorylation/dephosphorylation processes, which are mediated by protein kinases and protein phosphatases, play pivotal roles in the oocyte meiotic cell cycle. Maturation (or metaphase) promoting factor (MPF) and other cyclin-dependent kinases are key molecules in regulating cell cycle progression during both mitosis and meiosis. However, research during the last decade has revealed that the mitogen-activated protein kinase (MAPK) cascade is another principal regulatory system that functions parallel to and interacts with MPF in driving the meiotic cell cycle progression of oocytes.

MOS/MEK1/MAPK/p90rsk signaling pathway regulates the cell cycle through a cascade of protein kinase phosphorylation. MAPK, which is also termed extracellular-regulated kinase (ERK), is a family of Ser/Thr protein kinases that require dual phosphorylation on threonine and tyrosine residues to become fully activated. Two isoforms of MAPKs, ERK1 (p44) and ERK2 (p42), express widely in mammalian oocytes and play a pivotal role in meiosis [1]. MAPK kinase (otherwise known as MAPK-ERK kinase 1, MEK1), the direct activator of MAPK, is a dual-specificity protein kinase that activates MAPK through phosphorylation of the threonine-183 and tyrosine-185 within its activation loop [2]. MEK is also activated by phosphorylation, and its upstream activator in vertebrate oocytes is MOS, the product of the proto-oncogene *c-mos*. MOS is a 39-kDa germ cell-specific Ser/Thr protein kinase that was first identified in cells transformed by Moloney murine leukemia virus [3]. The *C-mos* mRNA is stored as maternal information in the growing oocytes and it is translated into protein, which initiates MAPK cascade phosphorylation during oocyte maturation [4]. The first found and best known physiological substrates of MAPK in oocytes is a 90-kDa protein kinase, p90rsk (ribosome S6 kinase), a family of Ser/Thr kinases that originally were cloned on the basis of their ability to phosphorylate the S6 protein of the 40S ribosomal subunit in maturing *Xenopus* oocytes [5]. P90rsk is activated by ERK1/2 in vitro and in vivo via phosphorylation on Ser369 and Thr577 [6].

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Evidence from *Xenopus* oocytes suggests that p90rsk mediates most of the MAPK function in regulating the meiotic cell cycle progression [7–9].

The regulation of MAPK cascade on the meiotic cell cycle has been well reviewed in recent years, but these reviews are based mainly on the findings in *Xenopus* oocytes [4, 10, 11]. Although the activation, localization, regulation, and function of MAPK cascade have been extensively studied in mammalian oocytes, controversial findings were reported in almost every step of the meiotic cell cycle. This minireview presents the results of recent findings in this area and, in particular, the current status of this research in mammals. Furthermore, based on the findings from our lab and from others, we attempted to highlight specific hypotheses that aim to explain the results concerning the roles of MAPK cascade during mammalian oocyte maturation and fertilization.

MAPK AND MEIOTIC RESUMPTION

Role of MAPK Cascade in Spontaneous and Induced Meiotic Resumption

In vivo, reinitiation of meiosis, signified by GVBD, is triggered by the pituitary LH surge, but the mechanism by which LH induces GVBD remains unknown. It has been suggested that meiotic resumption may result from release of inhibitory action imposed by the follicular environment, as a result of breakdown in gap junction communication between different follicle compartments and interruption of the action of cAMP or other inhibitory molecules on the oocyte. Alternatively, reinitiation of meiosis may be mediated by a positive signal able to override the conditions that ensure continued meiotic arrest [12, 13].

Basically, two in vitro experimental models have been employed for studying oocyte meiotic resumption in mouse and rat: the spontaneous meiosis model and the induced meiosis model. Although a hormone-induced mechanism leads to the meiotic resumption of mammalian oocytes under physiological conditions, the GV stage-arrested murine oocytes can also resume meiosis spontaneously when they are released from the inhibitory follicular environment. Alternatively, mouse and rat oocytes can mature in vitro under the stimulation of follicle-stimulating hormone (FSH) or epidermal growth factor when the spontaneous maturation is prevented by meiotic inhibitors, such as hypoxanthine (a natural meiotic inhibitory substance existing in follicular fluids [14]) and cAMP-elevating agents [15]. In these two events, different mechanisms are employed in regulating the progression of meiotic cell cycle. The former is thought to be a relatively passive response to the artificial removal from a meiosis-inhibiting environment of the follicles, whereas the latter is more active, presumably requiring gonadotropin-triggered production of a meiosis-inducing stimulus, supporting the view of the existence of an overriding stimulus [16]. Importantly, these ligands act through mediation by cumulus cells, as they fail to promote the same effect on denuded oocytes [16]. Furthermore, the mechanism of meiotic reinitiation may be different between rodents and domestic mammals. In farm animals such as pig, cattle, goat, and sheep, it is difficult for the oocyte to spontaneously enter meiosis unless physical or chemical stimulations are given. Protein synthesis is not required for spontaneous meiosis resumption in mouse and rat oocytes [17], while in pig, cow, sheep, and goat oocytes, protein synthesis is required for GVBD [18], as is the case in *Xenopus* oocytes [19].

The timing of activation of MAPK during oocyte maturation, unlike MPF, varies in mammalian species and in different maturation models. In some species, such as mouse and rat, MAPK (and its downstream kinase p90rsk) activation is not required for the initial activation of MPF and occurs 2 h after spontaneous GVBD [20–22]. However, in FSH-induced meiotic resumption of mouse oocytes in the presence of hepoxanthine, MAPK activity was detected at 8 h of FSH treatment, before GVBD that occurs approximately 12 h after culture [23, 24]. In oocytes from large domestic species, the temporal association between MAPK activation and GVBD is less definitive. In oocytes from pig, cow, goat, and horse, the cytoplasm is opaque and the nucleus is not visible in live cells. And due to the long duration of the GV stage (ranging from 8 h in bovine and ovine oocytes to 24 h in porcine oocytes) in maturation culture, the GVBD in these oocytes is somewhat asynchronous. Fissore et al. [25] reported that, in bovine oocytes, the activation of MAPK and MPF occurred simultaneously at 6 h of maturation culture, which is earlier than GVBD (which occurs approximately 8 h after culture). Whereas in goat oocytes, the appearance of MAPK activity (10–12 h after maturation culture) was delayed compared with MPF activity and the event of GVBD (8 h after culture) [26]. In pig oocytes, MAPK activity was low during the GV stage, 0–20 h of culture. Active MAPK was first detected at 18 h of culture, when GVBD occurs in a small number of oocytes, and an abrupt increase was observed at MI (30 h of culture) [27]. Similar patterns of MAPK activation were also described in mare, human, and rabbit oocytes [28–30].

P90rsk was partially phosphorylated in mammalian oocytes at the GV stage through a MAPK-independent mechanism, but its full phosphorylation after GVBD is dependent on MAPK activity. After the first appearance, concomitant with or shortly after GVBD, the activity of MAPK and p90rsk increases with the progression of meiosis I, culminating at MI stage and remaining high in MII-arrested oocytes in all mammalian species studied so far (mouse [31], rat [21], pig [32–34], and rabbit [30]).

Accumulating evidence suggests that MAPK activity is not required for the spontaneous meiotic resumption of denuded oocytes (DOs) in mammals. In spontaneous maturation of mouse and rat oocytes, MAPK is activated 2 h after GVBD [20–22]. In mouse DOs, GVBD occurred normally when MAPK activity was inhibited by the MEK inhibitor U0126 [34]. Furthermore, in *c-mos* knockout mice, oocytes could also resume meiosis, although MAPK failed to be activated in these cells [35, 36]. Studies in farm animals revealed similar results as in rodents. Microinjection of MKP-1 mRNA, which encodes a specific MAPK phosphatase, into GV-stage bovine oocytes could not prevent the meiotic resumption [37]. Microinjection of porcine *c-mos* antisense RNA into pig oocytes failed to arrest the cells at the GV stage [38]. And spontaneous meiotic resumption of pig DOs could occur normally when the MAPK phosphorylation was thoroughly inhibited by U0126 [32].

Interestingly, some recent experiments provided deeper insight into the roles of MAPK cascade in meiotic resumption of mammalian oocytes: MAPK activity in cumulus cells is necessary for the gonadotropin-induced meiotic resumption of oocytes. MEK inhibitors, U0126 and PD98059, inhibited FSH-induced meiotic resumption in cumulus-enclosed mouse and pig oocytes but not the spontaneous GVBD in denuded oocytes [23, 24, 32, 39]. MAPK was activated in mouse and pig cumulus cells promptly

TABLE 1. A summary of MAPK cascade functions in meiotic cell cycle progression of mammalian and amphibian (*Xenopus laevis* as the representative) oocytes.

Cell cycle stage	Functions of MAPK cascade	
	Mammals	*Xenopus*
Meiotic resumption	Necessary for FSH-induced meiotic resumption (functions in cumulus cells). Not necessary for spontaneous GVBD and MPF activation.	Facilitate progesterone-induced meiotic resumption (functions in oocytes). Involved in MPF activation.
GVBD-MI	Regulate normal meiotic spindle assembly.	Same as mammals.
MI-MII	Ensure asymmetric division of meiosis I. Not determined in S-phase inhibition.	Inhibit APC activity; prevent complete cyclin B degradation and S-phase entry of oocytes. Promote cyclin B synthesis.
MII arrest	Contribute to CSF activity; maintain MII arrest and normal spindle configuration.	Contribute to CSF activity. P90rsk is its sole mediator. Involved in spindle assembly checkpoint.
PB2 emission	Required for PB2 emission. Inhibit sperm aster development.	No reports.
PN formation	Incompatible with pronuclear membrane.	Same as mammals.

after FSH stimulation (0.5 and 2 h after FSH stimulation in mouse and pig cumulus cells, respectively). Activity of MAPK in pig cumulus cells increased following maturation culture and peaked at 20 h of culture [32, 40]. However, the regulation and functional roles of MAPK in cumulus cells is so far unknown. In recent years, cultured intact follicles have also been employed in the research of mammalian oocytes [41, 42]. Inhibition of MAPK activity prevents LH-stimulated meiosis resumption in mouse follicle-enclosed oocytes as well as the rise in expression of two genes, the products of which are necessary for normal cumulus expansion [43].

Considering the function and regulation of MAPK cascade in meiotic maturation of *Xenopus*, mouse, and pig oocytes/cumulus-oocyte complexes, a unified understanding emerged. The meiotic resumption of *Xenopus* oocytes is induced by progesterone, which exerts its effect on the oocytes directly and stimulates the MAPK cascade [4, 10, 11]. However, mammalian oocytes (especially rodent oocytes) can resume meiosis in two different ways: spontaneous meiotic resumption when released from inhibitory follicular environment, which is MAPK independent; and gonadotropin-induced meiotic resumption by overcoming the inhibitory effects of the follicular environment, which is MAPK dependent [23, 24, 32]. In the latter case, cumulus cells, instead of the oocytes, are the primary targets of meiosis-inducing signal (gonadotropin). Thus, the MAPK cascade facilitates meiotic resumption among all vertebrates in response to the maturation-inducing stimuli, either in oocytes or in cumulus cells, depending on the primary targets of the stimulations (for a summarization of MAPK functions in *Xenopus* oocytes and mammalian oocytes, see Table 1).

In mouse and rat oocytes, protein synthesis is required for activating MAPK but not cdc2/cyclin B kinase, even when phosphatases are inactivated by okadaic acid (OA), suggesting the existence of a positive control that may involve the synthesis of the proto-oncogene *c-mos* product [44].

The activity changes of MAPK and MPF during oocyte maturation and fertilization are summarized in Figure 1.

Crosstalk of MAPK with Other Protein Kinases During Meiotic Resumption

MAPK and MPF. In *Xenopus* oocytes, progesterone-dependent entry into meiosis I is accompanied by the synthesis of MOS [45, 46]. Accumulation of MOS above a threshold level activates the MAPK pathway [47], which leads to MPF activation, most likely through activation of p90rsk. Activated p90rsk inactivates Myt1, a negative regulating kinase of MPF, thereby promoting activation of MPF and entry into meiosis I [48]. Experimental treatments that block activation of MAPK inhibit GVBD significantly [49, 50]. In *Rana* (another amphibian species) oocytes that lack pre-MPF, injection of *c-mos* mRNA failed to induce GVBD [51]. This result further supports the idea that MAPK cascade facilitates GVBD by activating the pre-MPF. However, there are also other pathways that are involved in the activation of MPF during meiotic resumption. It has been well known in *Xenopus* oocytes that polo-like kinase Plx1 is able to phosphorylate and activate the phosphatase Cdc25C, leading to dephosphorylation of p34cdc2 and activation of MPF [52]. The involvement of polo-like kinase-1 (Plk1) in mammalian oocyte meiosis has also been reported recently [53–56], but the contribution of Plk1 to the MPF activation during mammalian oocyte maturation needs further study.

The fact that MPF regulation in mouse oocytes is not mediated by MOS/MAPK is supported by studies on oocytes derived from *c-mos* knockout mice, which display a normal pattern of MPF activation [44, 57, 58]. However, knowledge about the possible regulation of MPF on MAPK activation during meiotic maturation is limited. A recent report showed that in spontaneously maturing rat oocytes, MPF inhibitor roscovitine prevented the synthesis of MOS and subsequently the activation of MAPK [59].

Although MAPK activity is not necessary for spontaneous GVBD in mammalian oocytes, there is evidence that an artificial increase in MAPK activity accelerates GVBD. In bovine oocytes, injection of MOS mRNA elicited a rapid activation of MAPK that resulted in accelerated resumption of meiosis [25]. In porcine oocytes at the G2/M transition stage, active MAPK and p90rsk moved into the GV just before GVBD [32, 60] and the active MAPK injected into the GV markedly accelerated GVBD [59]. Treatment of the mouse, rat, and pig oocytes with OA, a phosphatase 1 and 2A inhibitor, resulted in the prompt activation of MAPK and precocious GVBD [22, 61, 62]. These results suggest that, although in natural conditions MAPK is not involved in the initiation of MPF activation during meiotic resumption, artificial activation of MAPK in premature mammalian oocytes may lead to MPF activation and GVBD by an undefined mechanism. However, earlier activation of MAPK by treatment with OA did not lead to premature spindle formation but instead an interphase network of microtubules developed with time [63]. Thus, MAPK is unable to substi-

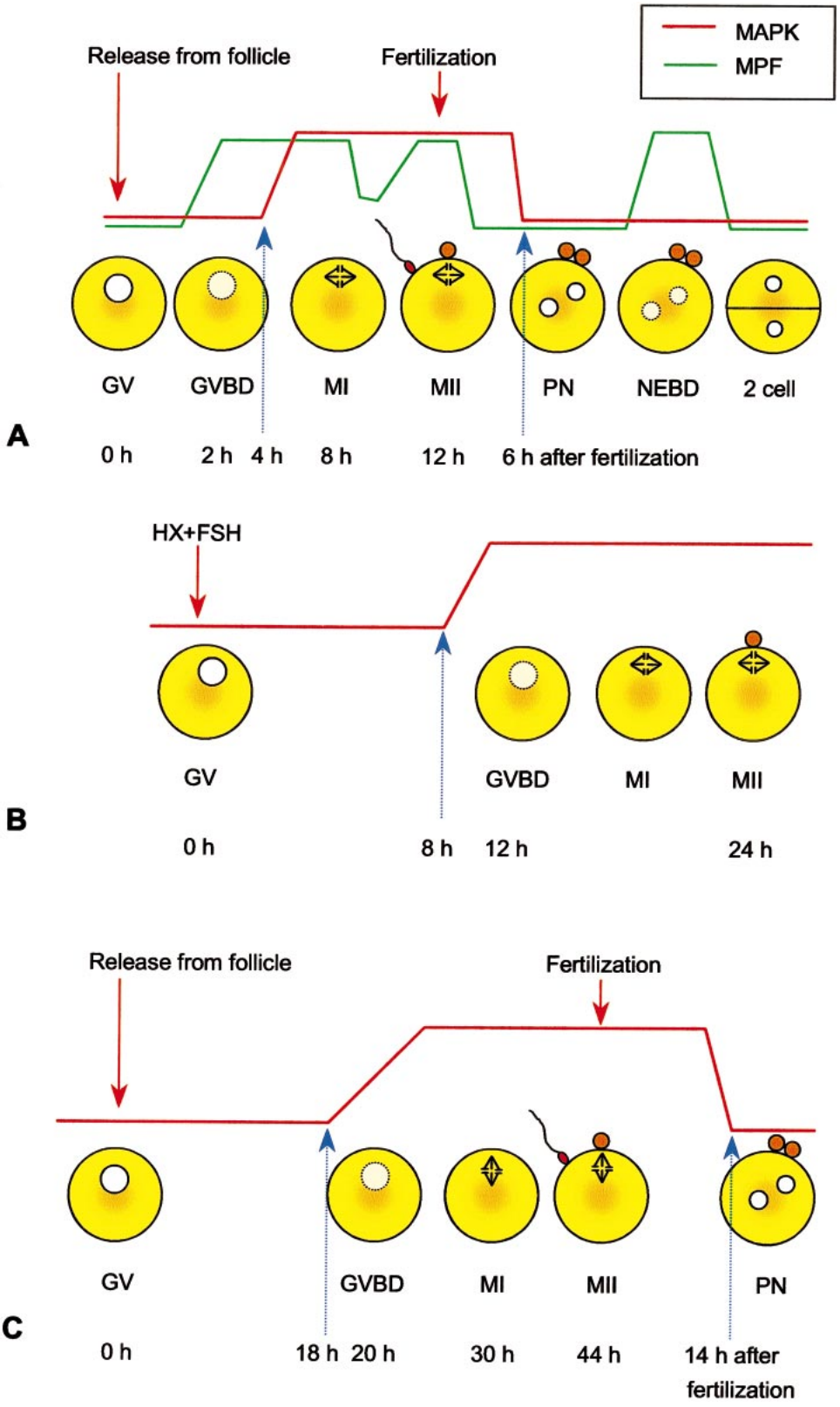

FIG. 1. The change of MAPK activity during mammalian oocyte maturation and fertilization. (**A**) Activity changes of MAPK (expressed by red line) and MPF (expressed by green line) in spontaneous meiotic maturation and fertilization of rodent (mouse and rat) oocytes. MAPK is activated 2 h after GVBD and is inactivated at approximately 6–8 h after egg activation, either by parthenogenetic stimulation or in vitro fertilization. MAPK keeps high activity between the two meioses, whereas MPF activity drops at the transition from MI to MII. (**B**) Activity changes of MAPK in FSH-induced meiotic maturation of mouse oocytes, which were cultured in medium containing 4 mM hypoxanthine and 100 IU FSH. MAPK is activated at 8 h after culture and shortly before GVBD in oocytes. (**C**) Activity changes of MAPK in meiotic maturation and fertilization of pig oocytes, which were cultured in medium containing gonadotropin but not meiotic inhibiting substances. MAPK is activated simultaneously with the occurrence of GVBD at 18 h after maturation culture. The inactivation of MAPK occurs at approximately 14 h after egg activation, shortly before pronuclear formation.

tute for MPF and its activity alone is insufficient to maintain the progression through meiotic maturation.

MAPK and cAMP/PKA. A drop in intracellular cAMP levels followed by the decrease of the cAMP-dependent protein kinase A (PKA) activity is associated with resumption of meiosis. GVBD can be blocked by the addition of dibutyryl cyclic AMP (dbcAMP), a membrane-permeable cAMP analogue, isobutyl-methyl-xanthine (IBMX), a phosphodiesterase (PDE) inhibitor, or forskolin, an adenylate cyclase activator. However, which mechanism initiates GVBD after inactivation of PKA is still not clear. We probed the relationship between MAPK phosphorylation and cAMP pathway in mouse, rat, and porcine oocytes [21, 64, 65]. The results showed that, when dbcAMP, IBMX, or

forskolin was added in the culture medium to increase the intracellular cAMP level, both GVBD and MAPK activation were inhibited. If the drugs were added after GVBD, MAPK activation as well as the following meiotic events such as first polar body emission and MII spindle formation were not affected. These effects suggest that perhaps the decrease of cAMP levels in GV oocytes is the premise of MAPK activation [66]. However, cAMP pathway may block GVBD by inhibiting MPF activation rather than inhibiting MAPK phosphorylation because MAPK activation is not the prerequisite of spontaneous meiotic maturation in mammalian oocytes.

How does the cAMP/PKA pathway inhibit MAPK activation? We found that, if OA was added to the culture medium, the blockage of GVBD and MAPK activation by PKA activation was overcome. Calphostin C, an inhibitor of protein kinase C (PKC), could partially reverse the effect of dbcAMP on oocytes. These facts suggest that, in the cAMP/PKA pathway, an OA-sensitive protein phosphatase may mediate the inhibition of MAPK activation. Furthermore, PKC may also be involved in the cAMP-mediated MAPK activity regulation [66].

MAPK and PKC. PKC plays a role in the regulation of meiotic maturation in both oocytes and cumulus cells. Intriguingly, their effects on meiotic resumption appear to be controversial in the spontaneous and the induced oocyte maturation models. The profiles and subcellular localizations of PKC isozymes were investigated in mouse, rat, and pig oocytes. The presence of eight PKC isoforms, cPKCα, βI, and γ; nPKCδ, ϵ, and μ; and aPKCζ and λ, was shown in rat oocytes [67], and cPKCα, βI, and βII; nPKC δ; a-PKCλ and ζ existed in mouse oocytes [68–71]. We also reported the expression of cPKCα, βI, and γ proteins in pig oocytes [72]. Results from our lab and others indicated that PKC activators, such as phorbol 12-myristate 13-acetate (PMA) and 1-oleoyl-2-acetyl-sn-glycerol (OAG), significantly inhibited the spontaneous meiotic resumption in mouse [64], rat [21], and pig oocytes [65]. The same studies showed that activation of PKC down-regulated MAPK phosphorylation and inhibited meiosis resumption in denuded rodent oocytes [21, 64], and these effects were overcome by the protein phosphatase (PP) inhibitor OA. Thus PKC activation might inhibit meiosis resumption and MAPK phosphorylation at a step before OA-sensitive protein phosphatases.

Regarding the interaction between MAPK and cAMP/PKA or PKC signaling pathway, it has been suspected that the inhibitory effects of PKC activators on MAPK activation is just the result of their inhibition on GVBD, and there is in fact no crosstalk between PKC and MAPK. A recent report argued that MAPK activation was independent of the germinal vesicle material during porcine oocyte maturation [73]. This is also the case in mouse oocytes. MAPK activation in enucleated mouse oocytes could also be inhibited by PKC activation (unpublished results from our lab). These results indicate that an interaction between PKA or PKC and MAPK does exist in the cytoplasm regardless of the inhibition of GVBD. Further research is necessary to identify the components responsible for the inhibition of MAPK phosphorylation in the cytoplasm of mammalian oocytes.

It has been recognized that PKC activators inhibit spontaneous GVBD in DOs but stimulate the meiotic resumption in cumulus-enclosed oocytes (CEOs) through the mediation of cumulus cells [74], but the knowledge about the signal transduction process of PKC in cumulus cells that leads to oocyte meiotic resumption is not clear. We provided evidence that PKC activators, phorbol 12-myristate 13-acetate (PMA) and 1-oleoyl-2-acetyl-sn-glycerol (OAG), could activate MAPK in cumulus cells in the absence of FSH stimulation, and MEK inhibitor U0126 efficiently prevented the PMA- or OAG-induced GVBD of CEOs. PKC inhibitors, calphostin C and chelerythrine, blocked the FSH-induced oocyte meiotic resumption and MAPK activation. FSH and PKC activators induced GVBD in denuded oocytes cocultured with cumulus cells in HX-supplemented medium, and this effect could be reversed by U0126 (see Note Added in Proof). Based on these findings, we hypothesized that PKC might be the linkage between FSH stimulation and MAPK activation in induced meiosis resumption of mouse CEOs. When activated by FSH and PKC, MAPK may stimulate the synthesis of specific proteins and then the secretion of a certain meiosis-activating substance by cumulus cells. This hypothesis is also supported by other reports. Su et al. [75] suggested that PKC and intracellular calcium (which is a well-known activator of conventional PKC isoforms) are involved in FSH-mediated GVBD of pig CEOs cultured in HX-supplemented medium. Similarly, inhibition of phosphoinositide metabolism or chelation of intracellular calcium could also block FSH-induced meiotic resumption of mouse CEOs [13].

MAPK AND SPINDLE ORGANIZATION

The resumption of oocyte meiotic maturation is accompanied by extensive reorganization of the microtubular network. In interphase, long and relatively stable microtubules radiate throughout the cytoplasm. During mitosis, by contrast, microtubules are shorter and less stable and are concentrated at the region of the spindle. During mitosis and particularly during metaphase centrosomes, the microtubule organizing centers (MTOCs) that nucleate microtubules, become phosphorylated and microtubule-nucleating activity increases. These changes in MTOC activity may contribute to the changes in microtubule dynamics at metaphase.

There is evidence suggesting that the MAPK pathway is involved in the regulation of microtubule organization during mammalian oocyte meiosis. MAPK is present in the spindle and is particularly associated with the MTOCs present at the spindle poles as well as in the cytoplasm [76]. In mouse and pig oocytes, the active MAPK distributes to the spindle poles at metaphase and migrates to the middle of the spindle at anaphase. During polar body emission, MAPK is associated with the cytokinetic ring [77, 78]. The best known MAPK substrate in vertebrate oocytes, p90rsk, exhibits the same pattern of subcellular distribution as MAPK in pig oocytes during meiotic maturation [32]. This pattern of distribution makes MAPK a candidate for playing a determining role in spindle assembly and microtubular configurations. Activation of MOS/MAPK cascade in mouse oocytes leads to partial chromosome condensation and to the formation of microtubule arrays when MPF activity is inhibited [58]. Mouse oocytes injected with antibody against MOS failed to assemble a meiotic spindle [79], and the spindle shape was altered in maturing *mos*$^{-/-}$ oocytes [57, 58]. Reports in other mammalian species also suggested important roles of MAPK in microtubule organization. Bovine oocytes injected with MKP-1 mRNA, a MAPK-specific phosphatase that inhibits MAPK activation, exhibited disorganized and diffused spindles [37]. MAPK was colocalized with γ-tubulin in pig oocytes, suggesting that MAPK is involved in microtubule nucleation [80]. If

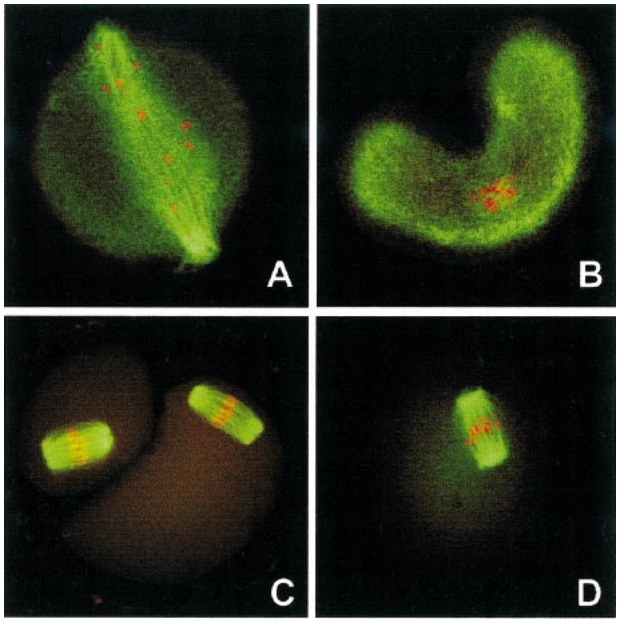

FIG. 2. Microtubule dynamics in the mouse oocytes with symmetric division after U0126 treatment. **A**) An MI spindle elongated extraordinarily (8 h after culture); (**B**) an oocyte undergoing symmetric division (8 h after culture); (**C**) each daughter cell formed a normal MII spindle (12 h after culture); and (**D**) a normal spindle in an oocyte at MI stage without U1026 treatment (8 h after culture). Original magnification × 630.

the activity of MAPK was inhibited by U0126, chromosome separation, first polar body emission, and MII spindle formation were also inhibited [78]. However, it was reported that mouse oocytes deficient in MAPK activity could still divide and release polar bodies [35, 36, 81].

In both vertebrates and invertebrates, meiotic divisions of oocytes are typically asymmetric, resulting in the formation of a large oocyte and small polar bodies. The size difference between daughter cells is a consequence of asymmetric positioning of the spindle before cytokinesis. Spindle movements are typically due to interactions between spindle asters and the cell cortex. In wild-type mouse oocytes, the meiotic spindle forms at the center of the cell and migrates to the cortex just before polar-body extrusion. The spindle does not elongate during anaphase. But the first meiotic division is symmetric in some $mos^{-/-}$ oocytes [58]. In $mos^{-/-}$ oocytes or $mos^{+/+}$ oocytes treated with U0126, the spindle forms centrally and elongates but does not migrate to the cortex, resulting in the formation of an abnormally large polar body [81, 34]. Some spindles in U0126-treated oocytes elongated extraordinarily at metaphase I/anaphase I transition and formed an astonishing large structure transversing the entire oocyte [34]. This indicates that the MOS/MAPK cascade in mouse oocytes plays a crucial role in normal spindle and chromosome morphology. The abnormal spindle organization in meiosis I after U0126 treatment is shown in Figure 2. The migration of the first meiotic spindle depends on microfilaments and not microtubules. This can be shown by treating oocytes with either cytochalasin D or nocodazole [82, 83]. These results demonstrate that the MAPK cascade might also control the activity of the microfilament network, perhaps through myosin IIA, a molecule involved in spindle migration [84]. However, the targets of MAPK on the meiotic spindle remain unclear. Recently, using two-hybrid screens, two new MAPK substrates were identified from a mouse cDNA library. One is named MISS (MAPK-interacting and spindle-stabilizing protein). This protein accumulates only in MII, where it localizes to the spindle. The depletion of endogenous RNA encoding for MISS induces severe spindle defects specific to MII oocytes [85]. Another MAPK partner, DOC1R, a murine homologue of a potential human tumor suppressor gene, is regulated by phosphorylation during meiotic maturation by MAPK pathway. DOC1R also localizes to microtubules and the injection of its antisense RNA leads to microtubule defects in MII oocytes [86].

MAPK is important for the establishment and maintenance of the spindle assembly checkpoint after microtubule depolymerization in *Xenopus* egg extracts. It was shown that immunodepletion of endogenous MAPK from interphase extracts prevents the establishment of the spindle checkpoint, and the checkpoint could be rescued by adding back recombinant MAPK [87]. In some mammalian epithelial cell lines, active MAPK, which accounts for only a minute fraction of the total MAPK present in the cell, was found on spindle poles and kinetochores. The staining of active MAPK on kinetochores of metaphase chromosomes was greatly enhanced after activation of the spindle assembly checkpoint by the microtubule depolymerizing drug nocodazole. Expression of MAPK phosphatase was able to abolish the nocodazole-induced arrest [88, 89]. In mammalian oocytes, the involvement of MAPK in the establishment of spindle checkpoint has not yet been determined, but some clues exist indicating that MAPK might play the same role in regulating spindle checkpoint as in *Xenopus* oocytes. As referred to above, the shape of the spindle was often severely distorted in oocytes from *c-mos* knockout mice or mice treated with U0126, but the first meiotic division was completed and a first polar body was emitted in most of these oocytes [31, 46], suggesting that, in the absence of MAPK activity, the oocytes failed to arrest the cell cycle at metaphase when the spindle is distorted. Sun et al. [90] investigated MAPK phosphorylation in relation to nuclear and cytoskeletal behavior during pig oocyte maturation and fertilization and found that chromosome condensation and metaphase microtubule assembly were temporally correlated with MAPK phosphorylation. MAPK phosphorylation still occurred when the oocytes were treated with cytochalasin B or nocodazole, although spindle formation was inhibited in the latter case. Treatment of either MI or MII oocytes with the microtubule-stabilizing drug taxol, although inducing changes in microtubule organization, did not increase MAPK phosphorylation levels. Further study is necessary to reveal the possible direct relationship between MAPK cascade and the spindle assembly checkpoint in mammalian oocytes.

MAPK AND MI-MII TRANSITION

The hallmarks of meiosis in the oocytes are the two consecutive divisions in the absence of S phase, resulting in one haploid germ cell. The period between the two meiotic divisions is defined as interkinesis, during which the oocyte is prevented from entering interphase. The activity of MPF declines during the transition between the two meiotic divisions, whereas the activity of MAPK is sustained. Formerly, it has been postulated that the sustained MAPK activity between the two meiotic divisions prevents DNA replication. This notion was fortified by experimental evidence showing that, in *Xenopus* oocytes, the mere ablation of MOS (either by using the antisense strategy or by microinjection of antibodies) results in the formation of a nucleus and replication of DNA [91]. Early experiments in mouse oocytes using the same techniques seem to indicate that,

without MOS, the nucleus is reformed and the DNA replicates, similar to *Xenopus* oocytes [92–95]. However, use of *c-mos* knockout mice, generated more recently, disclosed that, contrary to the above reports, interkinesis occurs in the absence of MAPK activity [35, 36]. Moreover, inhibition of MAPK did not interfere with the progression to the second meiotic metaphase in mouse and rat oocytes [34, 59].

One mechanism that normally ensures alteration of metaphase and anaphase is the degradation of B-type cyclin after ubiquitination by the anaphase-promoting complex (APC), an E3 ubiquitin ligase [96]. But in vertebrate oocytes, only partial cyclin degradation occurs during anaphase I. The residual MPF activity is essential for progression of *Xenopus* oocytes to meiosis II without interphase because inhibition of cyclin B synthesis at the MI-MII transition leads to complete degradation of cyclin B and entry into S phase [97]. In rat oocytes, when the reactivation of MPF at completion of the telophase I was prevented, the meiotic cell cycle failed to proceed to meiosis II. Instead, oocytes exhibited an interphase nucleus with decondensed chromosomes. In *Xenopus*, the ability of APC to degrade cyclin B is reduced by MAPK-dependent activation of p90rsk [9]. Further study suggests that p90rsk is sufficient not only to partially inhibit APC activity but also to stimulate cyclin B synthesis in meiosis II [97]. Whether this takes place in mammalian oocytes needs further study.

MAPK AND MII ARREST

In most vertebrates, unfertilized eggs are arrested at second meiotic metaphase by a cytostatic factor (CSF), which is defined as the activity capable of inhibiting the transition from metaphase II to anaphase II in mature oocytes [98]. CSF activity prevents ubiquitin-dependent degradation of metaphase cyclins and thus inactivation of MPF. CSF was first described in amphibian oocytes by Masui in the 1970s and it is not a specific molecule but represents many molecules. Some of them have been identified, while some are still unclear (for a review, see [10]). In *Xenopus* oocytes, thus far, the molecular nature of CSF activity appears to be downstream of the MOS/MEK/MAPK/p90rsk pathway. Each member of this pathway is synthesized and/or activated upon meiotic maturation of the oocytes, and each kinase depends only on downstream molecules of the pathway for the CSF-mediated establishment of MII arrest. Although there is no direct evidence that proves the existence of CSF activity in mammalian oocytes, a similar molecular basis might be responsible for the maintenance of MII arrest. In mammalian oocytes, only a requirement for MOS has thus far been firmly established. *Mos*$^{-/-}$ mouse oocytes undergo GVBD and progress through the first meiosis (although sometimes the first meiotic division is abnormal) but fail to arrest in MII. Instead, they undergo spontaneous parthenogenetic activation [35, 36]. Similarly, inhibition of MOS synthesis in mouse oocytes using RNA interference also resulted in parthenogenetic activation [99].

As in *Xenopus* and other nonmammalian species, fertilization and the release of MII arrest in mammalian oocytes induce the inactivation of MAPK [73, 100–102]. While MAPK inactivation occurs after MPF inactivation and thus does not mediate the release from MII arrest, it does appear necessary for the maintenance of MII arrest. MEK inhibitor U0126 parthenogenetically activates mouse oocytes [34, 103]. When porcine oocytes matured in vitro were further cultured in medium containing U0126, both the activities of MAPK and MPF were clearly reduced and nearly a quarter [102] to half [32] of the oocytes entered into interphase with pronuclear formation. From these findings, it is clear that MAPK activity is necessary for the maintenance of MPF activity and for the prevention of MII arrest release of mature oocytes.

In oocytes, cyclin B is to some extent in a balance of degradation and synthesis even at metaphase II. One explanation for the CSF-arrested state is that the MAPK-stimulated process prevents active APC from ubiquitinylating its substrates and/or MAPK-dependent protein synthesis overweighs ubiquitin-dependent degradation. An interesting fact is that, although CSF activity maintains the MII arrest, CSF or MAPK inactivation is not necessary for the release of MII arrest after fertilization. Under physiological conditions, release from MII arrest is mediated by Ca^{2+}/calmodulin-dependent kinase II (CaMKII), which triggers degradation of M-phase cyclins and sister chromatid segregation [104–107]. In other words, if MAPK inhibits cyclin degradation, CaMKII can overcome it. However, several puzzles still exist. MAPK cascade has already been fully activated at MI in mammalian oocytes but MAPK activity does not arrest meiosis at MI, as is the case in meiosis II. The question remains of what mechanism is responsible for the exit of MI with the presence of high MAPK activity. Furthermore, which mechanisms inhibit its function continually in MII? It appears that the MAPK pathway is not the only factor that contributes to CSF activity, and other unknown factors must be involved in the MII arrest. These factors, which aid the oocyte in completing CSF activity, may be synthesized between MI-MII transition, considering that the MAPK pathway has been activated completely before MI while meiosis was not arrested at MI.

It is argued that MAPK cascade may maintain the CSF arrest of oocytes by activating the molecules downstream of spindle assembly checkpoint (such as Bub1/3) in *Xenopus*, i.e., MAPK cascade utilizes the same molecules to maintain metaphase arrest as spindle checkpoint [10]. But there is still no experimental evidence to show that the same mechanisms are also used in mammalian oocytes. A very recent report showed that the spindle checkpoint component Bub1 localizes to kinetochores and is phosphorylated until anaphase of both meiotic M phases in mouse oocytes, but neither the kinetochore localization nor phosphorylation of Bub1 depends on the MAPK cascade [108].

ROLES AND REGULATION OF MAPK AFTER FERTILIZATION

Roles of MAPK after Fertilization

PB2 emission. The decrease in MPF activity precedes the decrease in MAPK activity after egg activation, but the cellular functions of this delayed inactivation of MAPK are still unclear. Incubation of MII-arrested oocytes in medium containing U0126 induced pig and mouse oocytes to complete the second meiotic division and interphase transition as indicated by pronucleus (PN) formation, but not second polar body emission. Electrostimulation caused the inactivation of both MPF and MAPK in pig oocytes, and the inactivation of MPF preceded the inactivation of MAPK. A sustained high level of MAPK activity was detected during the period of PB2 emission. However, the time sequence required for MAPK inactivation was significantly reduced by the addition of U0126 to the culture medium following electrostimulation. In these oocytes, PB2 emission was markedly inhibited but little difference was found

in the time course of PN formation compared with oocytes that were not treated with U0126 [102]. These findings suggest that the decrease in MAPK activity is partly involved in driving matured oocytes out of metaphase and in inducing PN development and that the delayed MAPK inactivation after the onset of MPF inactivation in activated oocytes has a crucial role for PB2 emission.

Mos$^{-/-}$ oocytes not only activate spontaneously but the parthenogenotes also exhibit a distinctive set of phenotypes, with a high proportion exhibiting abnormally large polar bodies and/or unequal and premature cleavages [109]. Absence of MAPK activity in mature MII oocytes and during egg activation is thought to affect the metaphase spindle and disrupt regulation of the unequal cytokinesis, which produces polar bodies. U0126 parthenogenetically activates mouse oocytes and produces phenotypes similar to *mos*$^{-/-}$ parthenogenotes (premature, unequal cleavages, and large PB2) [103]. This is consistent with results obtained in invertebrates such as *Urechis caupo*, oyster, and starfish, where inhibition of MAPK activity during fertilization results in large polar bodies and abnormal spindles [110, 111].

Pronuclear formation. Fertilization of the mammalian egg causes a wave of calcium ions spreading throughout the cell from the site of sperm entry. The transient increase in intracellular Ca^{2+} serves to activate the egg and causes the resumption of meiosis II. The egg is released from the CSF-mediated metaphase arrest and progresses from metaphase II to anaphase II, followed by the emission of the second polar body and entry into embryonic cell cycles. Following either fertilization or parthenogenetic activation in mouse [76, 112], bovine [100, 101], and porcine [113] oocytes, inactivation of MPF was found to occur very rapidly whereas a long time sequence was required for dephosphorylation/inactivation of MAPK. According to Liu et al. [100], the decrease in MPF activity is involved in the initiation of egg activation, i.e., the exit from MII, whereas the decrease in MAPK activity correlates with onset of pronuclear formation, showing that inactivation of MPF and MAPK probably occurs via two independent processes. The delayed inactivation of MAPK compared with MPF after egg activation will be reflected by the potent upstream functions of MOS, because cyclin subunits of MPF are degraded before MOS is degraded after fertilization, and thus, MPF is inactivated before CSF during activation of *Xenopus* oocytes [114].

Although MAPK cascade inactivation does not mediate the release from MII arrest, it does appear to regulate pronuclear formation. Parthenogenetic activation of mouse oocytes can be induced by various chemicals, including ethanol, calcium ionophore A23187, protein kinase inhibitors staurosporine, Rp-31-8220, genistein, and protein synthesis inhibitor cycloheximide. In these approaches, MAPK activity decreased abruptly before pronucleus formation [115, 116]. The protein phosphatase inhibitor OA was found to inhibit PN formation by preventing the decrease in MAPK activity in mammalian oocytes. Treatment of fertilized mouse and pig eggs that contain pronuclei with OA does not activate cdc2/cyclin B kinase but does activate MAPK by maintaining it in its phosphorylated state and results in the precocious breakdown of the pronuclear envelopes [51, 99]. Moos et al. [117] expressed in mouse eggs a constitutively active form of MEK, whose only known target is MAPK, and showed that, following fertilization, MPF activity declined and a second polar body was emitted, but no pronuclear envelope formed. Similarly, inactivation of MAPK after egg activation is associated with the formation of microtubular networks and PN development in bovine and pig oocytes [51, 88]. Thus, it is likely that MAPK activity by itself in mammalian eggs appears incompatible with the presence of pronuclear envelopes. However, although MAPK activity in pig oocytes treated with U0126 declined immediately after electrical stimulation, PN formation in these oocytes was not accelerated in comparison with untreated oocytes [102]. These findings suggest that MAPK activity may not be qualitatively essential for regulating the critical period necessary to undergo PN formation. Other factors are likely to be involved.

Sperm aster inhibition. In invertebrate eggs in which fertilization occurs before meiosis is completed, the sperm centriole-centrosome complex that is associated with the nucleus waits at the egg periphery until after polar body formation, when it nucleates a large aster that moves the sperm nucleus to join the haploid egg nucleus. Even in mammals, in which eggs are in MII when fertilization takes place, large sperm asters are not formed until after second polar body formation [118, 119]. During an investigation of MAPK activation in eggs of the marine worm *Urechis caupo* (which are in meiotic prophase at the time of fertilization), oyster, and starfish, Gould and Stephano [110, 111] discovered that, when MAPK activation was inhibited by the MEK inhibitors PD98059 or U0126, large sperm asters formed at the same time as the egg asters during meiosis, whereas normally they did not appear until after second polar body formation. Is MAPK suppression of sperm aster development during meiosis a universal mechanism? Indirect evidence from mammals is consistent with this idea. In all mammalian species studied so far, MAPK dephosphorylation has been reported to coincide with pronuclear formation. Why active MAPK persists when it is no longer needed to maintain metaphase arrest is largely unexplained; suppression of sperm aster growth might be the reason. The regulation of aster formation by centrosomes is not well understood in any cell type [120] and oocytes are ideal material for such studies because sperm and egg centrosomes behave differently in the same cytoplasm. A summary of MAPK cascade functions in mammalian and *Xenopus* oocytes is shown in Table 1.

Regulation of MAPK Activity after Egg Activation

Roles of MPF. The evolutionarily conserved stimulus that releases the egg from cell cycle arrest in all animal species studied so far is a rise in intracellular free Ca^{2+}, which is triggered by fertilization or parthenogenetic activation [121]. A not fully defined APC-dependent mechanism activated by Ca^{2+} inactivates MPF and releases oocytes from meiotic metaphase arrest.

U0126-induced parthenogenetic activation is dependent on an intact MII spindle and MPF inactivation. Disruption of the MII spindle has been shown to maintain MII arrest by preventing MPF activity for an extended time even after stimulation by processes such as fertilization [122]. Parthenogenetic activation induced by U0126 was essentially eliminated in the presence of democolcine, which disrupted the MII spindle and maintained the high MPF activity. In mouse and rat mature oocytes, inhibitors of ubiquitin-proteasome pathway, ALLN and MG-132, significantly inhibit cyclin B degradation, MAPK inactivation, and pronuclear formation induced by calcium ionophore A23187 and fertilization ([123], Huo et al., unpublished results). On the contrary, inactivation of MPF by roscovitine results in the

inactivation of MAPK and parthenogenetic activation with a time course similar to that following fertilization. Notably, roscovitine also produced some *mos*$^{-/-}$-like phenotypes, indistinguishable from U0126 parthenogenotes [103].

Roles of CaMKII. The MII arrest of mammalian oocytes is maintained by the activity of both MPF and MOS/MEK/MAPK/p90rsk cascade [4]. After egg activation, MPF is quickly inactivated by a calcium-induced and APC-dependent degradation pathway, which is the prerequisite for metaphase/anaphase transition [124]. However, the MAPK phosphorylation level and the amount of this kinase remain stable after egg activation until the initiation of pronuclear formation. It is not fully known by which mechanisms MPF is inactivated by calcium transients and how the MAPK activity is sustained after egg activation. In both mouse and pig oocytes, the fertilization- or parthenogenetic stimulus-induced degradation of cyclin B, the regulatory subunit of MPF, as well as the decrease of MPF activity are significantly inhibited by both calmodulin antagonist W7 and CaMKII inhibitors, KN-93 or AIP, suggesting that CaMKII, after activation by calcium and calmodulin (CaM), may facilitate anaphase transition by activating a cyclin B proteolysis mechanism [104–107]. CaMKII could also serve to potentiate MAPK and p90rsk activity after egg activation. Suppression of CaMKII activity during egg activation resulted in reduction in the amount and kinase activity of MAPK [77, 107]. Studies on mouse and pig oocytes revealed that MAPK and CaMKII were colocalized at the meiotic spindle, suggesting their potentially close mutual regulation [77, 107]. CaMKII could serve to potentiate MAPK activity and the colocalization of these two kinases suggests that such an interaction is facilitated. In combining these results, the authors hypothesize that CaMKII might play dual roles in mediating calcium signal after egg activation: on one hand, it inactivates MPF and releases the oocytes from MII arrest by stimulating a cyclin B degradation pathway; but on the other hand, CaMKII maintains the phosphorylated state and the level of MAPK/p90rsk through an unknown mechanism. The two key protein kinases controlling meiotic cell cycles may be differently regulated by the same calcium modulator, CaMKII.

Roles of PKC. As mentioned above, the rise in intracellular Ca^{2+} levels is necessary for oocyte activation, but the downstream molecular mechanisms are still not well known. Recently, PKC drew a lot of attention as another possible target of Ca^{2+} along with CaMKII. We found that PKC activators, PMA and diC8, induced parthenogenetic activation with pronuclear formation in mouse and rat mature oocytes, and this effect could be reversed by PKC inhibitors, staurosporine, or calphostin C [64, 125]. Because both PKC activation and MAPK inactivation are correlated with pronuclear formation during egg activation, the question is whether MAPK activity is regulated by PKC. Results in our lab showed that MAPK dephosphorylation was induced by both PMA and diC8 in a dose-dependent and time-dependent manner. Also, MAPK dephosphorylation was consistent with pronuclear formation when the mature oocytes were treated with PKC activators. This effect of PKC activators could be overcome by PKC inhibitors. These data suggest that PKC activation may induce pronuclear formation via a cascade that dephosphorylates MAPK in mouse and rat oocytes. Recently, Ito et al. [126] compared the role of a Ca^{2+} rise and PKC activation with the decrease of MPF and MAPK activity during parthenogenetic activation of pig oocytes. Treatment with high concentrations of calcium ionophore decreased both MPF and MAPK activity and induced pronuclear formation. However, a supplement of PKC inhibitor calphostin C was sufficient to inhibit MAPK inactivation and PN formation after Ca^{2+} ionophore treatment. Moreover, PMA treatment without Ca^{2+} ionophore treatment effectively led to a decrease of MAPK activity in a dose-dependent manner but not the MPF activity in mature pig oocytes. Thus, they concluded that the parthenogenetic activation of porcine oocytes was mediated by the inactivation of MPF via a calcium-dependent pathway and thereafter by the inactivation of MAPK via a PKC-dependent pathway [126]. One will remember that PKC activators inhibit MAPK activation and GVBD in the GV oocytes. Here in MII oocytes, PKC activator induced pathenogenetic activation as well as the inactivation of MAPK. These results suggest that PKC always down-regulates the activity of MAPK in mammalian oocytes at meiosis.

REGULATION OF PROTEIN PHOSPHATASE ON MAPK ACTIVITY IN MAMMALIAN OOCYTES

The importance of protein kinases and protein phosphorylation in the regulation of oocyte meiosis has been well recognized. However, its reverse process, i.e., the protein dephosphorylation mediated by protein phosphatases is also an important pattern of meiotic cell cycle regulation in mammalian oocytes. Serine/threonine protein phosphatases PP1 and 2A have been identified in mouse oocytes. They are involved in the regulation of MAPK activity and microtubule organization [127]. PPs inactivate MAPK by dephosphorylating the Thr residue of ERK1/2. OA, the inhibitor of PP1 and 2A, could completely reverse the inhibitory effect of PKA and PKC on GVBD and MAPK activation in mouse oocytes. Based on this fact, we hypothesize that the inhibitory effect of PPs on MAPK is downstream to cAMP/PKA [65, 66]. OA greatly accelerates GVBD and induces the prompt phosphorylation of MAPK and p90rsk in GV-arrested oocytes. In pig oocytes treated with OA, the MAPK was activated within 5 min, even when MPF activity was inhibited in the same cell [62]. This fact implied that fully grown GV oocytes have acquired the ability to phosphorylate MAPK/p90rsk, but there is an OA-sensitive phosphatase-dependent inhibitory mechanism that slows down the MAPK/p90rsk activation. OA induces MAPK activation and GVBD by overcoming the effects of this mechanism. Our hypothesis was supported by the reports of Inoue et al. [60], who found that active MAPK injected into cytoplasm of pig GV oocytes was quickly dephosphorylated and failed to induce GVBD. Though it is MPF, instead of MAPK, that initiates the G2/M transition in mammalian oocytes, OA treatment leads to the preterm activation of MAPK and initiates meiotic resumption by a redundant pathway.

OA also leads to the disturbance of microtubule organization in MII mouse and rat oocytes [63]. In fertilized or parthenogenetically activated oocytes, OA induces pronuclear membrane breakdown, chromatin condensation, and the reactivation of MAPK and p90rsk [32, 62]. The regulation of MAPK and p90rsk inactivation after egg activation has been poorly understood up to now, but this evidence suggests that, even in activated eggs with PN formation, the machinery responsible for MAPK activation still exists. The inactivation of MAPK cascade may be the result of enhanced activity of OA-sensitive phosphatase before PN formation. Because OA also blocks protein synthesis, long-time OA treatment may lead to the decrease of

MAPK quantity. Arrighi et al. [128] and Lu et al. [127] reported in mouse oocytes that a pulse of OA treatment (1 h) induced the irreversible activation of MAPK and thus may avoid the apparent toxic effects. Although this approach was not tried in pig oocytes, the results from authors suggested that the changes of MAPK phosphorylation and cell cycle progression after long-term OA treatment [32, 62] were similar to those reported in mouse oocytes after short-term OA treatment.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Although considerable similarities are evident between the functions of the MAPK cascade in *Xenopus* and mammals, there are nevertheless fundamental differences. For example, although the activation of the MOS/MEK1/MAPK/p90rsk cascade around the time of GVBD is a feature of meiotic maturation, MAPK activation is required for GVBD in *Xenopus* oocytes, while spontaneous GVBD and meiotic maturation can occur in mammals in the complete absence of MAPK activation. However, recent evidence suggests that MAPK activity in cumulus cells is indispensable for gonadotropin-induced meiotic resumption of mammalian oocytes. Does the molecular basis of induced meiotic maturation in mammalian oocytes resemble that in *Xenopus* oocytes, or does the MAPK cascade function by totally different ways in the two cases? Correct chromosome cohesion and separation are essential for both mitosis and meiosis. Although poorly investigated, the regulation of chromosome cohesion and separation in meiosis is likely to be more complicated in oocytes at meiosis than during mitosis, considering that sister chromatids remain in cohesion in meiosis I but are separated at anaphase II. The key protein that regulates the dissolution is a cysteine protease known as separase [129]. In mitotic S phase and metaphase, separase activity is doubly inhibited by a chaperone protein securin and phosphorylation at multiple sites [130, 131]. It is evident that MAPK can phosphorylate separase in vitro. Is MAPK also involved in the regulation of separase activity and then chromosome separation during the two meiotic anaphases? Furthermore, only the involvement and possible roles of MAPK cascade in oocyte meiosis are discussed in this minireview. But the expression and phosphorylation of MAPK were also detected during spermatogenesis, which is the meiosis event of male germ cells [132]. Prominent expression of p90rsk is also observed in spermatocytes by immunohistochemistry (unpublished observation). Does MAPK cascade also play key roles in the meiosis of male germ cells, and if so, does it play the same or different roles as in the case of oocyte meiosis? Further study concerning MAPK in spermatogenesis will undoubtedly deepen the understanding of meiosis-regulating mechanisms in an extensive sense.

Taken together, MAPK and MPF, the two important protein kinases for oocyte meiosis, interact intimately and perform essential regulating roles in meiotic maturation and fertilization. Despite the fact that oocytes from different animals arrest and fertilize at different stages of the cell cycle, the activation and inactivation of MAPK follow the same rule. Its activity emerges and vanishes concurrently with chromatin condensation and is incompatible with the presence of an intact nuclear membrane. Although p90rsk appears to be downstream of MAPK in several meiotic processes, our knowledge about the substrates of MAPK cascade is limited, which highlights a recurrent problem in signal transduction biology: the difficulty of identifying genuine in vivo biochemical targets of protein kinases in biological processes.

NOTE ADDED IN PROOF

During production of this paper, previously unpublished results referenced here have been published [133].

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