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Phosphorylation of Mitogen-Activated Protein Kinase Is Regulated by Protein Kinase C, Cyclic 3′,5′-Adenosine Monophosphate, and Protein Phosphatase Modulators During Meiosis Resumption in Rat Oocytes[1]

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ABSTRACT

Mitogen-activated protein (MAP) kinase, protein kinase C (PKC), cAMP, and okadaic acid (OA)-sensitive protein phosphatases (PPs) have been suggested to be involved in oocyte meiotic resumption. However, whether these protein kinases and phosphatases act by independent pathways or interact with each other in regulating meiosis resumption is unknown. In the present study, we aimed to determine the regulation of meiosis resumption and MAP kinase phosphorylation by PKC, cAMP, and OA-sensitive PPs in rat oocytes using an in vitro oocyte maturation system and Western blot analysis. We found that ERK1 and ERK2 isoforms of MAP kinases existed in a dephosphorylated (inactive) form in germinal vesicle breakdown (GVBD)-incompetent and GVBD-competent germinal vesicle intact (GVI) oocytes as well as GVBD oocytes at equivalent levels. These results indicate that MAP kinases are not responsible for the initiation of normal meiotic resumption in rat oocytes. However, when GVBD-incompetent and GVBD-competent oocytes were incubated in vitro for 5 h, MAP kinases were phosphorylated (activated) in GVBD-competent oocytes, but not in meiotic-incompetent oocytes, suggesting that oocytes acquire the ability to phosphorylate MAP kinase during acquisition of meiotic competence. We also found that both meiosis resumption and MAP kinase phosphorylation were inhibited by PKC activation or cAMP elevation. Moreover, these inhibitory effects were overcome by OA, which inhibited PP1/PP2A activities. These results suggest that both cAMP elevation and PKC activation inhibit meiosis resumption and MAP kinase phosphorylation at a step prior to OA-sensitive protein phosphatases. In addition, inhibitory effects of cAMP elevation on meiotic resumption and MAP kinase phosphorylation were not reversed by calphostin C-induced PKC inactivation, indicating that cAMP inhibits both meiotic resumption and MAP kinase activation in a PKC-independent manner.

cAMP, gametogenesis, kinases, meiosis, oocyte development, phosphatase, signal transduction, ovum

INTRODUCTION

During oogenesis oocytes gradually acquire competence to undergo meiotic maturation. Fully grown (meiotic competent) but not growing (meiotic incompetent) mammalian oocytes can spontaneously resume meiosis in vitro upon their release from antral follicles [1]. A cascade of protein phosphorylation/dephosphorylation events, which are regulated by protein kinases and protein phosphatases (PPs), regulates meiotic arrest and resumption. It is well known that oocyte meiosis resumption is associated with decreased concentration of intracellular cAMP and resulted inactivation of cAMP-dependent protein kinase A (PKA) [1, 2]. Another protein kinase, calcium/phospholipid-dependent protein kinase C (PKC) is also involved in meiosis resumption of oocytes. PKC activators have been shown to inhibit germinal vesicle breakdown (GVBD) in denuded mouse oocytes [3–5]. However, activation of PKC stimulates meiotic maturation of follicle-enclosed oocytes in rat and bovine [6, 7]. Regulation of oocyte meiosis by PPs has also been investigated. Continued inhibition of oocyte PP1, PP2A, or both, with okadaic acid (OA) stimulates GVBD, but induces cytoplasmic aberrations and spindle abnormalities, and thus inhibits progression to metaphase II (MII) [8–10].

Recently, the roles of mitogen-activated protein (MAP) kinases in oocyte maturation have received much attention. MAP kinases are a family of serine/threonine protein kinases that are activated by MEK (MAP kinase kinase) phosphorylation of both tyrosine and serine/threonine residues [11, 12]. Both ERK1 and ERK2, the two main isoforms of MAP kinases, are activated around or after GVBD during mammalian oocyte maturation [8, 13–17]. It has been suggested that MAP kinase is not implicated in meiosis resumption, but rather in post-GVBD events in mouse, rat, porcine, and goat oocytes [8, 14–16, 18–20]. However, injection of active MAP kinase or overexpression of MAP kinase by injecting either oncoprotein MOS or MEK RNA in mouse, bovine, and porcine oocytes induced GVBD, indicating that these protein kinases may promote meiotic resumption under special conditions [17, 21, 22]. MAP kinases have also been found to mediate precocious GVBD of meiotic-incompetent mouse oocytes by OA [23]. These results suggest that MAP kinases may not be directly necessary for normal resumption of meiosis, but precocious activation of MAP kinases does induce meiosis resumption. In addition, our previous study has shown that the ability of cytoplasmic factors to phosphorylate MAP kinase is a

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prerequisite for resumption of meiosis in mouse oocytes [24].

Thus, it is evident that cAMP, PKC, MAP kinase, and PPs are key molecules in regulation of oocyte meiotic cell cycle progression. However, questions remain as to whether these molecules act by independent pathways or interact with each other in regulating meiosis resumption. Our recent studies have shown that MAP kinase phosphorylation is down-regulated by either cAMP elevators or tumor promoting phorbol ester during mouse oocyte maturation [24, 25]. In the present study, we aimed to study the cross-talk among these signal molecules during rat oocyte meiotic maturation.

MATERIALS AND METHODS

Oocyte Collection and Culture

Fully grown germinal vesicle-intact (GVI) oocytes were collected from 30-day-old rats of the Sprague-Dawley strain by puncturing the antral follicles in M2 medium (Sigma Chemical Company, St. Louis, MO). Growing GVI oocytes were collected from 20-day-old rats of the same strain by puncturing the follicles in the same medium. Cumulus cells surrounding the oocytes were removed by repeated pipetting. The denuded oocytes were cultured in M2 medium containing 4 mg/ml BSA at 37°C, 5% CO_2 in air.

Chemicals

Stock solutions of phorbol 12-myristate 13-acetate (PMA, 1.62 mM), 4α-phorbol 12,13-didecanoate (4α-PDD, 1.62 mM), 1,2-dioctanoyl-rac-glycerol (diC8, 100 mM), calphostin C (Cal, 0.5 mM), okadaic acid (OA, 0.2 mM), forskolin (10 mM), and isobutylmethylxanthine (IBMX, 50 mM) were prepared with dimethyl sulfoxide. Dibutyryl cAMP (dbcAMP, 10 mM) was diluted in M2 medium. All chemicals used in this study were purchased from Sigma Chemical Company (St. Louis, MO) unless otherwise noted. All stock solutions were stored frozen at −20°C. Prior to use, they were diluted with M2 medium containing 4 mg/ml BSA.

Experimental Design

Experiment 1. The kinetics of MAP kinase phosphorylation during rat oocyte maturation was evaluated. Denuded GVI oocytes from 30-day-old ovaries were cultured in M2 medium containing 4 mg/ml BSA for 2 h. The GVBD oocytes were selected and further cultured for an additional 0, 2, 4, 6, 8, 10, 12, or 14 h, and then collected and lysed for detecting the phosphorylated (active) MAP kinase by Western blot analysis using anti-active MAPK antibody, which recognizes dually phosphorylated ERK1 and ERK2. MAP kinase phosphorylation in GVBD-incompetent oocytes, including growing oocytes from 20-day-old ovaries and those remaining at GVI stage after 2 or 5 h of incubation of GVI oocytes from 30-day-old ovaries, was also investigated. The expression of MAP kinase in these oocytes was also identified by Western blot analysis using anti-ERK2 antibody, which recognizes both active and inactive ERK2 and ERK1. Oocyte stages (GVI, GVBD, and MII) were evaluated with an inverted phase-contrast microscope. Metaphase I (MI) stage of oocytes was judged by immunocytochemistry analysis using anti-β-tubulin anti-

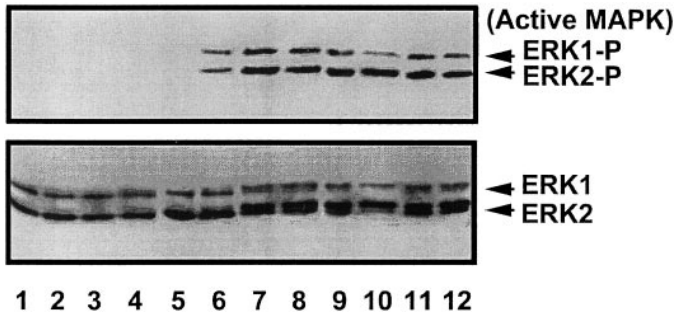

FIG. 1. MAP kinase expression and phosphorylation during rat oocyte maturation in vitro. The top panel shows phosphorylated (active) ERK1 and ERK2 detected by Western blot analysis using anti-active MAPK antibody. The bottom panel shows both active and inactive ERK1 and ERK2 detected by Western blot using anti-ERK2 antibody. The same was adapted to Figures 3, 4, 6, and 8. Lane 1, GVBD-incompetent oocytes from 20-day-old rat ovaries; lane 2, GVBD-competent GVI oocytes from 30-day-old rats; lanes 3–4, oocytes from 30-day-old rat ovaries remained at GVI stage after 2 h and 5 h of incubation, respectively; lane 5, oocytes just underwent GVBD at 2 h of incubation; lanes 6–12, oocytes collected at 2, 4, 6, 8, 10, 12, and 14 h post-GVBD, respectively.

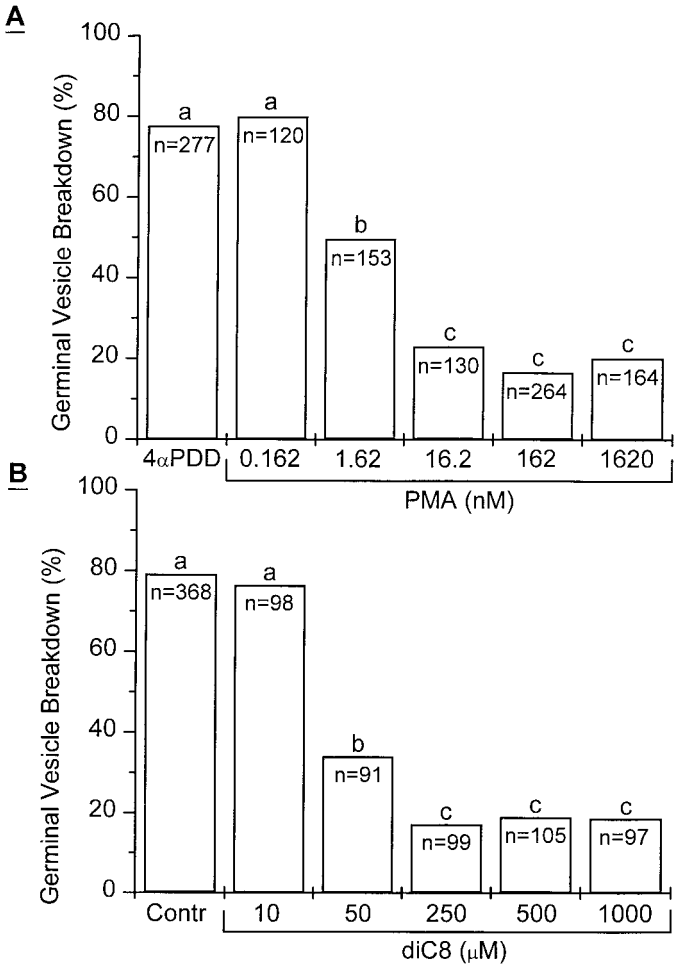

FIG. 2. Effects of PKC activators on GVBD in rat oocytes. The top panel (**A**) shows the effects of different concentrations of PMA on GVBD. The bottom panel (**B**) shows the effects of different dosages of diC8 on GVBD. Fully grown GVI oocytes were treated with different concentrations of PKC activators (PMA; 0.162, 1.62, 16.2, 162, and 1620 nM; diC8, 10, 50, 250, 500, and 1000 μM) for 5 h. As controls, GVI oocytes were treated with 4α-PDD (162 nM) or drug-free medium for 5 h. GVBD oocytes were identified at 5 h of incubation. Different letters mean statistically significant.

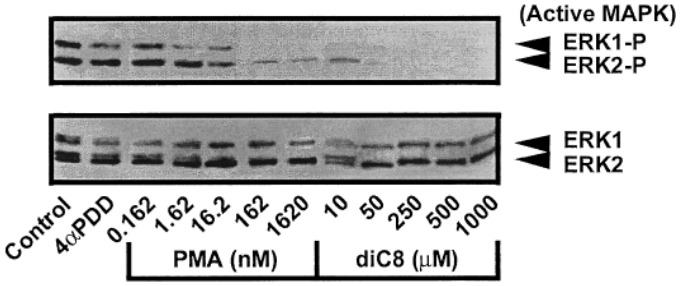

FIG. 3. Effects of PKC activators on MAP kinase phosphorylation in rat oocytes. GVI oocytes were subjected to different treatments for 5 h and collected for immunoblot. Lane 1, control, oocytes incubated in drug-free medium; lane 2, control, oocytes treated with 4α-PDD (162 nM); lanes 3–7, oocytes treated with 0.162, 1.62, 16.2, 162, and 1620 nM of PMA, respectively; lanes 8–12, oocytes treated with 10, 50, 250, 500, and 1000 μM of diC8, respectively.

body to detect spindles and Hoechst 33342 staining to detect chromosomes.

Experiment 2. To determine the role of PKC in rat oocyte maturation and MAP kinase phosphorylation, fully grown denuded GVI oocytes were treated with different concentrations of PMA (a pharmacological PKC activator, 0.162, 1.62, 16.2, 162, and 1620 nM) or diC8 (a physiological PKC activator, 10, 50, 250, 500, and 1000 μM) for 5 h. As controls, GVI oocytes were cultured in drug-free medium or treated with 4α-PDD, a biologically inactive phorbol ester, for 5 h.

Experiment 3. To determine the effects of treatment time of PKC activators on meiotic resumption and MAP kinase phosphorylation, fully grown denuded GVI oocytes were treated with PMA (162 nM) or diC8 (250 μM) for 10, 15, 30, and 60 min, respectively. After complete washing, they were transferred to a drug-free M2 medium and cultured for up to 5 h. As controls, GVI oocytes were cultured in drug-free medium or treated with 4α-PDD for 5 h. Germinal vesicle breakdown and MAP kinase phosphorylation were detected in all groups of oocytes at 5 h of incubation.

Experiment 4. To address the effects of cAMP on meiosis and MAP kinase phosphorylation in rat oocytes, fully grown denuded GVI oocytes were treated with dbcAMP (500 μM), forskolin (500 μM), or IBMX (1 mM) for 5 h. As a control, GVI oocytes were cultured in drug-free medium for 5 h. Meiotic progression and MAP kinase phosphorylation were analyzed.

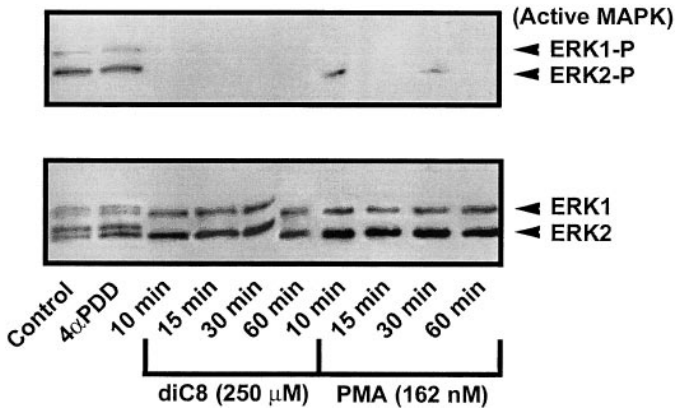

FIG. 4. Effects of treatment time of PKC activators on MAP kinase phosphorylation in rat oocytes. GVI oocytes were subjected to different treatments for up to 5 h and collected for immunoblot. Lane 1, control, oocytes incubated in drug-free medium for 5 h; lane 2, control, oocytes treated with 4α-PDD (162 nM) for 5 h; lanes 3–6, oocytes treated with 250 μM diC8 for 10, 15, 30, and 60 min, respectively, and then after complete washing they were transferred to drug-free medium and incubated for up to 5 h; lanes 7–10, oocytes treated with 162 nM PMA for 10, 15, 30, and 60 min, respectively, and then after complete washing they were transferred to drug-free medium and incubated for up to 5 h.

Experiment 5. Possible involvement of protein phosphatases in regulation of meiotic arrest and MAP kinase phosphorylation inhibition caused by cAMP elevators was studied. Fully grown denuded GVI oocytes were first treated with dbcAMP (500 μM), forskolin (500 μM), or IBMX (1 mM) for 10 min, and then transferred to a medium containing one of these former chemicals plus 2 μM OA, and cultured for an additional 5 h. Germinal vesicle breakdown and MAP kinase phosphorylation were identified in these treated oocytes.

Experiment 6. In order to further confirm that PKC is actually involved in the effect of PMA or diC8 on GVBD and MAP kinase phosphorylation, GVI oocytes were pretreated with PMA (162 nM) or diC8 (250 μM) for 10 min, and after complete washing, were cultured in medium containing calphostin C (a specific inhibitor of PKC, 1 μM) for an additional 5 h. As controls, GVI oocytes were treated with PMA (162 nM) or diC8 (250 μM) for 10 min, and after washing, were cultured in drug-free medium for an additional 5 h. To test whether PKC is involved in the inhibition of GVBD and MAP kinase activation caused by cAMP elevation, GVI oocytes were first treated with dbcAMP (500 μM), forskolin (500 μM), or IBMX (1 mM) for 10 min, and then transferred to a medium containing one of these former chemicals plus calphostin C (1 μM) for an additional 5 h. GVI oocytes were treated with PMA (162 nM) or diC8 (250 μM) for 10 min and then transferred to a medium containing one of the PKC activators plus 2 μM OA for up to 5 h to investigate the possible involvement of one or more PPs in regulation of meiotic arrest and MAP kinase phosphorylation inhibition caused by PKC activation. Germinal vesicle breakdown and MAP kinase phosphorylation were assessed.

All experiments were repeated at least three times.

Western Blot Analysis

Western blot analysis was conducted as previously described by us [24, 25]. Proteins extracted from 30 denuded oocytes were separated by 10% SDS-PAGE and electrophoretically transferred onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). After blocking, the membrane was incubated for 1 h at room temperature with rabbit polyclonal anti-Active MAPK antibody (Promega, Madison, WI; 1:1000 dilution), followed by 1 h incubation with horseradish peroxidase (HRP)-conjugated goat anti-rabbit immunoglobulin G (IgG; Jackson ImmunoResearch Laboratories, West Grove, PA; 1:5000 dilution). After washing, the membrane was processed using the enhanced chemiluminescence (ECL) detection system (Amersham International plc, Little Chafont, Buckinghamshire, England). MAP kinase expression was detected by reprobing the stripped membranes with rabbit polyclonal anti-ERK2 antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA; 1:300 dilution) and HRP-conjugated goat anti-rabbit IgG (1:5000 dilution). Image processing was conducted using Photoshop 5.0 software.

Data Analysis

All data were processed by χ^2 analysis. Differences at $P < 0.05$ were considered significant. Oocytes displaying signs of degeneration were excluded from analysis.

RESULTS

Kinetics of MAP Kinase Phosphorylation During Rat Oocyte Maturation in Vitro

Germinal vesicle-intact oocytes from 30-day-old rat ovaries can spontaneously resume meiosis in vitro. After incubation for 2 h, 63.1% (377/597) of GVI oocytes underwent GVBD. When GVBD oocytes were selected and incubated for an additional 5 h, 95.7% (177/185) reached MI stage. When GVBD oocytes were cultured for an additional 14 h, 81.2% (151/186) developed to MII stage. The kinetics of MAP kinase phosphorylation during rat oocyte maturation is shown in Figure 1 (top panel). MAP kinases were inactive in fully grown GVI oocytes. When GVBD occurred, they still remained inactive. MAP kinases were phosphorylated (activated) at 2 h following GVBD, and remained highly activated throughout the MI/MII transition. As in GVBD-competent GVI oocytes, active MAP kinases were also not detected in GVBD-incompetent oocytes, including those collected from 20-day-old ovaries and those remaining at GVI stage after 2 or 5 h of incubation of GVI oocytes from 30-day-old ovaries. The expression level of MAP kinases did not change during acquisition of meiotic resumption ability and maturation of oocytes (Fig. 1, bottom panel).

Effects of PKC activators, PMA and diC8, on GVBD and MAP Kinase Phosphorylation

Previous study has shown that PKC activators stimulate GVBD in rat follicle-enclosed oocytes [6]. Denuded oocytes were used in this study to eliminate the influence of cumulus cells on oocytes. As shown in Figure 2A, the PKC activator, PMA, inhibited GVBD in a dose-dependent manner in rat denuded oocytes, whereas the biologically inactive phorbol ester, 4α-PDD, had no effect. Similar results were obtained when a physiological PKC activator, diC8, was used instead of PMA (Fig. 2B). It is interesting that MAP kinase activation was also inhibited by both PMA and diC8 in a dose-dependent manner in denuded rat oocytes (Fig. 3). Inhibition of MAP kinase phosphorylation in oocytes treated with either PMA or diC8 was well correlated with inhibition of GVBD. Similar to its lack of effect on GVBD, 4α-PDD also had no effect on MAP kinase phosphorylation (Fig. 3).

Some PKC activators have been shown to up-regulate PKC activity with short-time treatment and to down-regulate PKC activity with long-time treatment [26]. In the present study, GVI oocytes were treated with PMA or diC8 for different times, and GVBD (results not shown) and MAP kinase phosphorylation (Fig. 4, top panel) were inhibited in a time-independent fashion (Fig. 4), whereas PKC activators exerted no effect on MAP kinase expression (Fig. 4, bottom panel).

Effects of cAMP Modulators on Meiotic Resumption and MAP Kinase Phosphorylation

As shown in Figure 5 (columns 1–4), cAMP elevators significantly inhibited GVBD. Correspondingly, increased cAMP concentration evidently inhibited MAP kinase phosphorylation (Fig. 6, top panel, lanes 1–4). However, cAMP modulators had no effect on MAP kinase expression (Fig. 6, bottom panel, lanes 1–4). The inhibitory effects of cAMP on GVBD and MAP kinase phosphorylation were abolished by OA (Fig. 5, columns 5–8; Fig. 6, lanes 5–8).

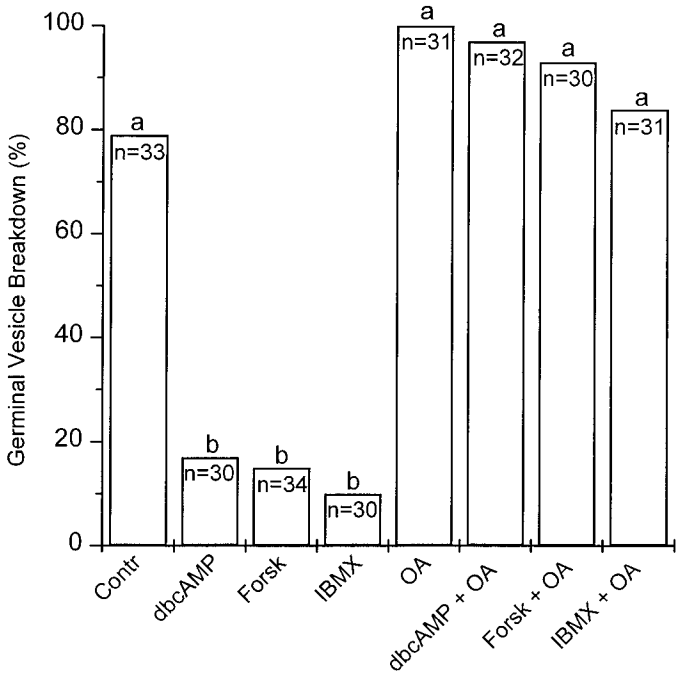

FIG. 5. Effects of cAMP elevators and OA on GVBD in rat oocytes. GVI oocytes were treated with cAMP elevators dbcAMP (500 μM), forskolin (500 μM), and IBMX (1 mM), or PP1/PP2A inhibitor OA (2 μM) alone or first treated with cAMP elevators (dbcAMP 500 μM, forskolin 500 μM, IBMX 1 mM) for 10 min and then treated with OA (2 μM) in the presence of one of these cAMP elevators for 5 h. As control, GVI oocytes were incubated in drug-free medium for 5 h. GVBD of all groups of oocytes was evaluated at 5 h of incubation. Different letters mean statistically significant.

Interaction of PKC, cAMP, and PP Modulators in Regulating Meiotic Resumption and MAP Kinase Phosphorylation in Rat Oocytes

Inhibition of GVBD and MAP kinase phosphorylation by PMA was reversed by calphostin C (Fig. 7, columns 1–3; Fig. 8, lanes 1–3). Similar results were obtained when diC8 was used instead of PMA (data not shown).

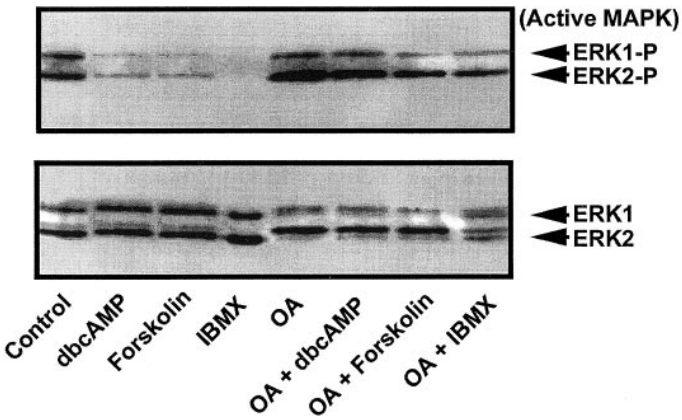

FIG. 6. Effects of cAMP elevators and OA on MAP kinase phosphorylation in rat oocytes. GVI oocytes were subjected to different treatments for 5 h and collected for immunoblot. Lane 1, control, oocytes incubated in drug-free medium; lanes 2–4, oocytes treated with cAMP elevators, dbcAMP (500 μM), forskolin (500 μM), and IBMX (1 mM), respectively; lane 5, oocytes treated with OA (2 μM); lanes 6–8, oocytes first treated with cAMP elevators (dbcAMP 500 μM, forskolin 500 μM, IBMX, 1 mM) for 10 min and then treated with OA (2 μM) in the presence of one of these cAMP elevators for 5 h.

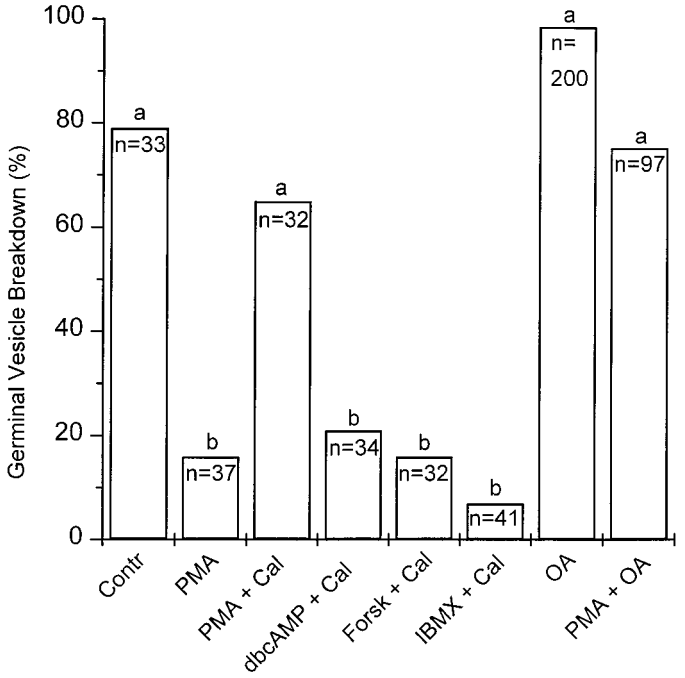

FIG. 7. Interaction of PKC, cAMP, and PP modulators in regulating GVBD in rat oocytes. GVI oocytes were treated with PKC activator PMA (162 nM) alone, or pretreated with 162 nM PMA for 10 min, and then after complete washing they were treated with 1 μM calphostin C for an additional 5 h; GVI oocytes were first treated with cAMP elevators (dbcAMP 500 μM, forskolin 500 μM, IBMX 1 mM) for 10 min, and then transferred to a medium containing one of these former chemicals plus calphostin C (1 μM) for up to 5 h; GVI oocytes were also treated with OA (2 μM) alone, or first treated with cAMP elevators (dbcAMP 500 μM, forskolin 500 μM, IBMX 1 mM) for 10 min and then treated with OA (2 μM) in the presence of one of these cAMP elevators for an additional 5 h. GVI oocytes incubated in drug-free medium for 5 h were designed as control. GVBD in all groups of oocytes was evaluated at 5 h. Different letters mean statistically significant.

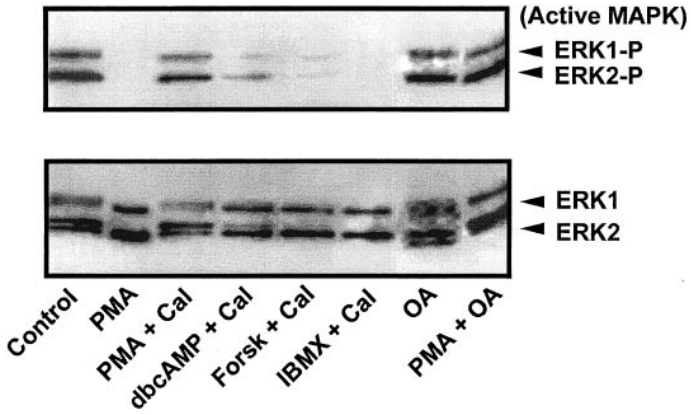

FIG. 8. Interaction of PKC, cAMP, and PP modulators in regulating MAP kinase phosphorylation. GVI oocytes were subjected to different treatments for 5 h and collected for immunoblot analysis. Lane 1, control, oocytes incubated in drug-free medium; lane 2, oocytes treated with PKC activator PMA (162 nM); lane 3, oocytes pretreated with 162 nM PMA for 10 min and then treated with 1 μM calphostin C for an additional 5 h; lanes 4–6, oocytes were first treated with cAMP elevators (dbcAMP 500 μM, forskolin 500 μM, IBMX 1 mM) for 10 min and then transferred to a medium containing one of these former chemicals plus calphostin C (1 μM) for up to 5 h; lane 7, oocytes treated with OA (2 μM); lane 8, oocytes first treated with cAMP elevators (dbcAMP 500 μM, forskolin 500 μM, IBMX 1 mM) for 10 min and then treated with OA (2 μM) in the presence of one of these cAMP elevators for up to 5 h.

Inhibition of PKC failed to reverse the inhibitory effect of cAMP elevators on GVBD (Fig. 7, columns 4–6) or on MAP kinase phosphorylation (Fig. 8, lanes 4–6). When oocytes were treated with PMA for 10 min and then transferred to the medium containing both PMA and OA for up to 5 h, inhibition of GVBD and MAP kinase phosphorylation was overcome (Fig. 7, columns 7–8; Fig. 8, lanes 7–8).

DISCUSSION

During the growth phase of oogenesis, oocytes gradually acquire the ability to undergo meiotic maturation. Rat oocytes do not acquire the competence to spontaneously resume meiosis until 22 days postpartum [27]. However, the molecular basis of this meiotic competence is unknown. MAP kinases have been suggested to be involved in OA-induced precocious GVBD in meiotic-incompetent mouse oocytes [23]. One of the aims of this study was to explore whether MAP kinases are responsible for acquisition of oocyte competence to spontaneously resume meiosis. Immunoblot analysis showed that GVBD-incompetent oocytes express ERK1 and ERK2 isoforms of MAP kinases in amounts that are not different from those found in GVBD-competent oocytes. Moreover, neither GVBD-incompetent nor GVBD-competent oocytes have active MAP kinases. They were activated 2 h after GVBD in fully grown oocytes. These data indicate that MAP kinases are not involved in normal meiosis resumption of rat oocytes. However, MAP kinases were activated only in GVBD-competent oocytes, but not in GVBD-incompetent oocytes, suggesting that oocytes acquire the ability to phosphorylate MAP kinase during the acquisition of meiotic competence.

Meiotic resumption is associated with decreased intracellular concentrations of cAMP and resulting inactivation of PKA [1, 2]. Two isoenzymes of PKA differentially regulate oocyte maturation. Mouse oocytes express only type I PKA, whereas cumulus cells express both type I and type II. Activation of type I maintains oocytes in arrest at the GV stage, whereas activation of type II results in GVBD in mouse oocytes [28]. In the rat, more than 95% of the PKA homoenzyme present in cumulus cells is type II [29]. In the present study, we confirmed that GVBD in denuded rat oocytes was inhibited by increasing the concentration of intraoocyte cAMP due to treatment with dbcAMP, forskolin, or IBMX. These data suggest that cAMP may inhibit GVBD by activating type I PKA, leading to increased phosphorylation of an unidentified inhibitory protein. We found it interesting that MAP kinase phosphorylation was also inhibited in these treated oocytes. It is well known that MOS is required for MAP kinase activation in oocytes [12, 18–21]. Bovine oocytes actively synthesize MOS at metaphase, whereas little synthesis is detected during the first 4 h of in vitro maturation when oocytes have not lost their germinal vesicles [30]. It has been suggested that some unidentified protein phosphorylated by PKA directly or indirectly suppresses c-mos translation [31, 32]. Therefore, MAP kinase activation is inhibited by these known elevators of intraoocyte cAMP, probably by mediation of type I PKA activation and the resulting decrease of MOS synthesis. How cAMP and activated PKA negatively control MOS synthesis and MAP kinase activation remains to be determined. In this study, we also found that OA reversed the inhibitory effect of cAMP on both GVBD and MAP kinase activation. These results suggest

that OA-sensitive protein phosphatases may down-regulate MAP kinase activation either at a step downstream to the cAMP/PKA pathway or by directly acting on MAP kinases.

Like PKA, PKC also has been suggested to inhibit resumption of meiosis in denuded mouse oocytes [12–14]. In contrast, PKC activators, phorbol 12-myristate 13-acetate (TPA) and 1-oleoyl-2-acetyl glycerol (OAG), stimulated GVBD in follicle-enclosed rat oocytes [6]. In the present study, we found that both PMA and diC8 inhibited GVBD and MAP kinase activation in a dose-dependent manner in denuded rat oocytes. Calphostin C, a specific PKC inhibitor, overcame the inhibitory effect of PMA on meiosis resumption and MAP kinase phosphorylation. Recent studies show that different isoforms of PKC exist in rat oocytes and follicular cells [33, 34]. Thus, it is logical to conclude that PKC activation down-regulates MAP kinase phosphorylation in rat oocytes. However, we should remember that different PKC isoforms require different cofactors and interact with different substrates, probably reflecting separate and even opposing roles upon their modulators [34–37]. Therefore, future work is needed to clarify the role of various PKC isoforms in MAP kinase regulation.

Our study also revealed that PKC, like cAMP, also negatively controls meiosis resumption and MAP kinase phosphorylation by mediation of OA-sensitive PPs. Because the inhibitory effects of cAMP elevators on meiosis resumption and MAP kinase phosphorylation were not reversed by PKC inhibitor, we suggest that increased cAMP concentrations in oocytes inhibit meiotic resumption and MAP kinase phosphorylation in a PKC-independent manner.

In summary, increased cAMP and PKC activation, acting through independent pathways, entrain a cascade of events, in which OA-sensitive PPs may be intermediate molecules, inhibiting meiotic resumption and MAP kinase phosphorylation during rat oocyte maturation.

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