Meiotic Arrest in Incompetent Rat Oocytes Is Not Regulated by cAMP

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Fully grown, but not growing, mammalian oocytes spontaneously resume meiosis *in vitro*. Resumption of meiosis, also known as oocyte maturation, is associated with a drop in intraoocyte concentrations of cAMP followed by activation of the maturation promoting factor (MPF). Microtubule-associated-protein (MAP) kinase has been suggested as a substrate for the active $p34^{cdc2}$ kinase, the catalytic subunit of MPF. Our study was designed to explore the mechanism of regulation of meiotic arrest in growing rat oocytes. Confirming previous observations we showed that in our rat colony oocytes do not acquire the competence to spontaneously resume meiosis earlier than 22 days postpartum. We further demonstrated that follicle-enclosed oocytes from 20-day-old female rats fail to resume meiosis in response to luteinizing hormone, follicle-stimulating hormone, a gonadotropin-releasing hormone analog, or forskolin, all of which are known to induce maturation in competent oocytes. Immunoblot analysis using highly specific anti $p34^{cdc2}$ antibodies revealed that incompetent oocytes express the catalytic subunit of MPF at amounts that are not different from that found in competent oocytes. In addition, highly specific anti MAP kinase antibodies detected the presence of similar quantities of two isoforms (42 and 44 kDa) of MAP kinase in competent and incompetent oocytes. Measurements of cAMP revealed that as compared to competent oocytes, incompetent oocytes contain somewhat lower levels of this nucleotide (1.42 ± 0.3 and 1.17 ± 0.07 fmole/oocyte, respectively). However, considering the difference in protein content, the calculated concentrations seem to be similar. Furthermore, similar to competent oocytes, intracellular concentrations of cAMP in incompetent oocytes dropped significantly (from 1.17 ± 0.07 to 0.77 ± 0.12 fmole/oocyte) 2 hr after isolation from the follicle. We hereby suggest that (a) in mammals, similar to amphibians, the term meiotic incompetence can be extended to include inability to resume meiosis in response to hormonal stimulation; (b) it is not the lack of $p34^{cdc2}$ or downstream regulatory elements, such as MAP kinase, that prevents growing oocytes from resuming meiosis; and (c) unlike fully grown oocytes, resumption of meiosis in growing oocytes is not subjected to negative regulation by cAMP.   © 1994 Academic Press, Inc.

INTRODUCTION

Oogenesis in mammals is initiated in the embryonic ovary. After several cycles of mitotic activity the ooge-nia are transformed into primary oocytes that embark on meiosis. Meiosis is arrested at birth and resumed after the onset of puberty, in a species-specific number of oocytes at each cycle. Resumption of meiosis, also known as oocyte maturation, is induced by the preovulatory surge of luteinizing hormone (LH)[1] and is associated with a drop in intraoocyte concentrations of cAMP (reviewed by Dekel, 1988). LH-induced resumption of meiosis can be demonstrated *in vitro* in follicle-enclosed oocytes (reviewed by Tsafriri and Dekel, 1994). Meiosis can also be spontaneously resumed *in vitro*, in oocytes released from the ovarian follicles (Pincus and Enzman, 1935). However, spontaneous maturation cannot be observed in oocytes isolated from female mice, hamster, and rats younger than 15, 23, and 22 days postpartum, respectively (Szybek, 1972; Iwamatsu and Yanagimachi, 1975; Bar-Ami and Tsafriri, 1981). Furthermore, in rodent females older than the above mentioned ages the competence to resume meiosis spontaneously is acquired only by those oocytes that have reached their final size (Iawamatsu end Yanagimachi, 1975; Sorensen and Wasserman, 1978; Bar-Ami and Tsafriri, 1981). Expression of meiotic competence which is correlated with an advanced stage of growth has also been reported for bovine and porcine (Motlik *et al.,* 1986), as well as for *Xenopus* oocytes (Sadler and Maller, 1983). Meiotically competent and incompetent oocytes are therefore referred to as fully grown and growing oocytes, respectively.

Balakier (1978) succeeded in inducing growing mouse oocytes to resume meiosis by their fusion with maturing, fully grown oocytes. This pioneering observation raised the idea that meiotic competence can be acquired experimentally upon transfer of the maturation promoting factor (MPF), which is present in an active form in the cytoplasm of maturing, fully grown oocytes. Later studies indeed demonstrated that resumption of meiosis can be induced in incompetent *Xenopus* oocytes upon mi-

[1] Abbreviations used: MPF, maturation-promoting factor; MAP, microtubule-associated protein; LH, luteinizing hormone; FSH, follicle-stimulating hormone; GnRHa, gonadotropin-releasing hormone analog; GV, germinal vesicle; GVB, germinal vesicle breakdown; IBMX, isobutyl-1-methylxanthine.

croinjection of a partially purified preparation of MPF (Hanocq-Quertier *et al.,* 1976; Sadler and Maller, 1983).

The present knowledge on the regulation of the meiotic cell cycle suggests that MPF is activated upon reinitiation of meiosis that entails a G_2/M transition of the cell cycle. An active MPF is a heterodimer composed of a dephosphorylated $p34^{cdc2}$ kinase and cyclin (reviewed by Norbury and Nurse, 1992). MAP kinase, which is known to phosphorylate the microtubule associated protein 2, has been implicated as a substrate for the $p34^{cdc2}$ kinase that is phosphorylated upon activation of MPF (Gotoh *et al.,* 1991).

Our study was designed in order to explore the mechanism of regulation of meiotic arrest in growing rat oocytes and to further understand the biochemical nature of acquisition of meiotic competence in mammals. Specifically, the following questions have been addressed: (1) Can resumption of meiosis in growing oocytes be induced by hormones? (2) Is the catalytic subunit of MPF, $p34^{cdc2}$, present in growing oocytes? (3) Do growing oocytes contain MAP kinase? and (4) Is meiotic arrest in growing oocytes negatively regulated by cAMP?

MATERIALS AND METHODS

Animals

Sexually immature Wistar female rat (19–25 days old) from our departmental colony were injected subcutaneously with 10 IU of pregnant mare's serum gonadotropin (Intervet, Holland) in 0.1 ml of 0.9% NaCl. The rats were sacrificed 24 hr later.

Culture of Follicles

The ovaries were removed and placed in Leibovitz's L-15 tissue culture medium (Gibco, U.S.A.), supplemented with 10% fetal bovine serum (Biolab, Israel), penicillin (100 IU/ml), and streptomycin (100 µg/ml, Gibco). This composition is referred to as control medium throughout this paper. Follicles were dissected under a stereoscopic microscope and transferred into 2 ml of the control medium in 25-ml flasks, gassed with 50% O_2 and 50% N_2. The incubations were carried out at 37°C in an oscillating water bath in the presence of the indicated concentrations of either forskolin (Sigma, U.S.A.), the gonadotropin-releasing hormone agonist analog D-Ser(t-Bu)6-des-Gly10-GnRH-*N*-ethylamide (GnRHa, Hoechst AG, Germany), follicle-stimulating hormone (FSH; NIH o-FSH-13) or LH (NIH o-LH-25). At the end of the incubation the follicles were incised and the cumulus-enclosed oocytes recovered. The oocytes were analyzed for maturation by Nomarski interference contrast microscopy. In the presence of the germinal vesicle (GV), oocytes were classified as meiotically arrested. Re-

sumption of meiosis was indicated by the absence of the GV in the individual oocytes (GV breakdown, GVB).

Culture of Oocytes

Cumulus–oocyte complexes released from the ovarian follicles were placed in 200-µl drops of control medium in 35-mm petri dishes. Incubations were carried out at 37°C in air at a relative humidity of 100% for 17 hr. At the end of incubation the oocytes were analyzed for maturation as described above.

Determination of cAMP in the Oocytes

Cumulus–oocyte complexes released from the ovarian follicles were transferred into control medium with or without 0.2 mM of isobutyl-1-methylxanthine (IBMX, Sigma). This concentration of IBMX prevents any drop in intraoocyte cAMP for at least 2 hr (Dekel, 1987). Collagenase (Sigma, 50 IU/ml) and EDTA (2.0 mM) at pH 7.2 were added and the cumulus cells were removed by several passages through a glass micropipet (100-µm diameter, approximately). After 2 hr incubation at 37°C, the cumulus-free oocytes were extensively washed (4×) and transferred with a minimum volume of medium. Sodium acetate buffer (50 mM, pH 6.5) containing 0.2 mM of IBMX was then added and repeated freezing and thawing (3×) on liquid nitrogen were used to release the oocyte cAMP content into the buffer. A similar volume of medium of the last wash was similarly treated to obtain blank values. The samples were kept at −80°C. For cAMP assay the samples were lyophilized and reconstituted with 300 µl of sodium acetate buffer (50 mM, pH 6.5). Intraoocyte cAMP was determined by cAMP ^{125}I radioimmunoassay (Harper and Brooker, 1975). The sensitivity range of the assay was 10–80 fmole/sample. An aliquot of a pooled sample of oocytes was included in each individual assay for normalization of interassay variations. Samples of extracts of cumulus-free oocytes, obtained after repeated freezing and thawing as described above, were subjected to protein determination (Bradford, 1976) and the protein content of a fully grown oocyte compared to a growing oocyte was calculated.

Antibodies

Antisera raised in rabbits against the PSTAIR peptide of $p34^{cdc2}$ were kindly provided by Professor Y. Yarden, The Weizmann Institute of Science, Rehovot, Israel. Polyclonal anti-MAP kinase antibodies that were raised against the peptide PRITVEEALAHPYLEQYY-DPTDE, which represents residues 307–327 of the *erk* gene product (Gause *et al.,* 1993), were kindly provided by Dr. R. Seger, University of Washington, Seattle, Washington.

Western Blotting

Extracts of cumulus-free oocytes were centrifuged at 1000 rpm for 2 min. The supernatant was removed and the pellet resuspended in lysis buffer (50 mM Hepes, pH 7.6, 150 mM NaCl, 1% Triton X-100, 1% SDS, 50 mM NaF, 10 mM sodium phosphate, 2 mM NaVO$_3$, 10 mM EDTA, 2 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, and 5 μg/ml leupeptin; Heffetz and Zick, 1989). The lysates were vortexed and incubated on ice for 5 min and then centrifuged for 5 min at 14,000g. The extracts were frozen on liquid nitrogen and kept in −80°C. Before electrophoresis, the extracts of each sample were mixed with concentrated (3×) electrophoresis sample buffer and heated to 90°C for 5 min.

For detection of either p34^{cdc2} or MAP-kinase, proteins were separated on 12.6% SDS–polyacrylamide gels using a mini gel apparatus (Bio-Rad, USA). At the end of electrophoresis the separated proteins were transferred to a nitrocellulose membrane (Hybond-C super; Amersham, UK) using a Milliblot Trans-blot apparatus (Bio-Rad) for 16 hr at 100 mA in transfer buffer (33 mM Tris, 192 mM glycine, 20% methanol). The membranes were then stained with Ponceau for normalization. For anti p34^{cdc2} immunoblot analysis, membranes were blocked with TTBS (10 mM Tris, pH 7.2, 0.9% NaCl, 0.1% Tween-20) containing 5% BSA, 1% ovalbumin, and 0.05% NaN$_3$ for 8 hr at 4°C. The membranes were then incubated with anti-p34^{cdc2} antibodies (1:500) overnight at 4°C, followed by three washes for 30 min in TTBS. For MAP-kinase immunoblot analysis, membranes were blocked with TTBS containing 5% low fat dry milk for 2 hr at room temperature. The membranes were then incubated for 2 hr at room temperature with the anti-MAP-kinase antibodies at a dilution of 1:1000 in the same buffer, followed by three washes for 30 min in TTBS. The blots were probed with the enhanced chemiluminescence (ECL) Western blotting detection system (Amersham) and were autoradiographed.

RESULTS

Our initial experiment was designed to determine in our rat colony the postnatal age when oocytes acquire the capacity to spontaneously resume meiosis *in vitro*. Confirming previous observations (Bar-Ami and Tsafriri, 1981) we found that oocytes recovered from 21-day-old rats or younger remained at the GV stage, whereas those from 22-day-old rats or older resumed meiosis at a frequency which increased with the age of the rat (Fig. 1). A maximal frequency of GVB oocytes was demonstrated in rats that were 25 days old. On the basis of these results 20- and 25-day old rats served as the source of incompetent (growing) and competent (fully grown) oocytes, respectively.
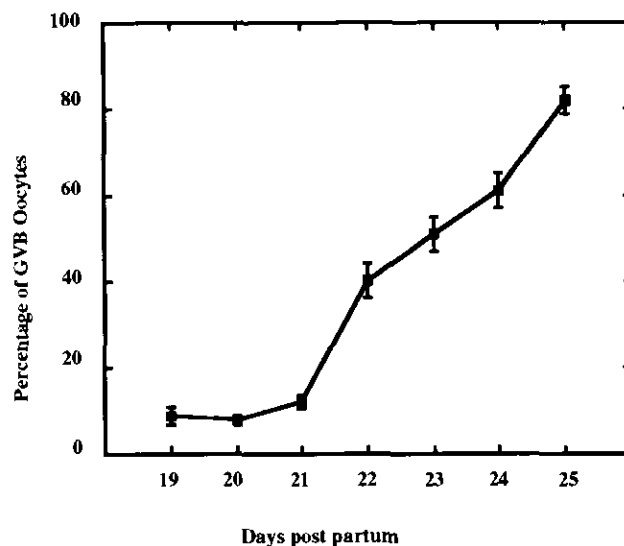
In mammals, meiotic incompetence has been defined

FIG. 1. Age-related incidence of meiotic competence in rat oocytes. Cumulus–oocyte complexes, recovered from the ovarian follicles of rats at the indicated ages postpartum, were incubated *in vitro* for 17 hr. At the end of the incubation the oocytes were examined for GVB as described under Materials and Methods. At least 250 oocytes were examined for each experimental point. Error bars indicate SEM.

so far as inability of the oocytes to resume meiosis spontaneously. We further tested the potential of growing rat oocytes to resume meiosis in response to hormonal stimulation. For this purpose follicle-enclosed oocytes recovered from 20-day-old female rats were incubated in the presence of either LH, FSH, GnRHa, or forskolin. Using similar culture conditions, we have previously shown that these agents successfully induced maturation in follicle-enclosed oocytes isolated from 25-day-old rats (Dekel *et al.*, 1983, 1988; Dekel and Sherizly, 1983). On the other hand, in the present study, we found that none of these agents could induce maturation in growing oocytes (Table 1).

TABLE 1
EFFECT OF OOCYTE MATURATION-INDUCING AGENTS
ON FOLLICLE-ENCLOSED OOCYTES FROM 20-DAY-OLD RATS

Treatment	Concentration	Fraction of GV oocytes (%)	Total No. of oocytes examined
—	—	99.1 ± 0.7	298
LH	5 μg/ml	89.2 ± 1.8	269
FSH	5 μg/ml	98.75 ± 0.4	488
GnRHa	10^{-7} M	97.1 ± 1.7	554
Forskolin	10^{-4} M	93.3 ± 2.4	258

Note. The presence of GV was assessed in oocytes isolated from ovarian follicles incubated for 17 hr in the presence of the indicated concentrations of LH, GnRHa, FSH, or forskolin.
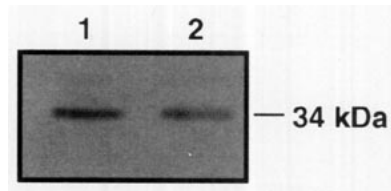
FIG. 2. Detection of p34^{cdc2} in incompetent rat oocytes. Lysates of either incompetent (428 oocytes, lane 1) or competent oocytes (300 oocytes, lane 2) were subjected to electrophoresis on a 12.6% acrylamide gel. Proteins were then immunoblotted with anti PSTAIR antibodies (1:500 dilution) followed by ECL detection. The resulting autoradiogram is shown.

The demonstration that growing oocytes resume meiosis following their microinjection by active MPF (Hanocq-Quertier et al., 1976; Sadler and Maller, 1983) raised the idea that this factor could possibly be absent at early stages of oocyte development. Immunoblot analysis with anti-p34^{cdc2} antibodies was performed for examination of growing rat oocytes for the presence of p34^{cdc2}, the catalytic subunit of MPF. Extracts of growing and fully grown oocytes isolated from 20- and 25-day-old female rats, respectively, were prepared and similar quantities of protein (428 growing and 300 fully grown oocytes/lane) were analyzed. We found that p34^{cdc2} is present in the growing oocytes (Fig. 2). Furthermore, the intensity of the bands detected by immunoblot analysis indicated that the intraoocyte level of p34^{cdc2} present in the growing oocyte is similar to that in fully grown oocytes.

The demonstration of two MAP kinase isoforms in fully grown mouse oocytes has been reported recently (Sobajima et al., 1993). In order to confirm that these kinases are also expressed in fully grown rat oocytes and to further analyze their presence in growing oocytes immunoblot analysis using anti-MAP kinase antibodies has been performed. Figure 3 demonstrates that the two isoforms (42 and 44 kDa) of MAP kinase are expressed in competent as well as incompetent rat oocytes. The amount of these proteins in the two groups of oocytes is not different.
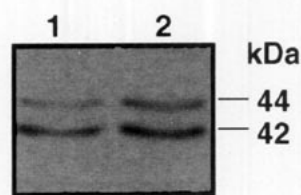

FIG. 3. Detection of MAP kinases in incompetent rat oocytes. Lysates of either incompetent (428 oocytes, lane 1) or competent oocytes (300 oocytes, lane 2) were subjected to electrophoresis on a 12.6% acrylamide gel. Proteins were then immunoblotted with anti MAP kinase antibodies (1:500 dilution) followed by ECL detection. The resulting autoradiogram is shown.
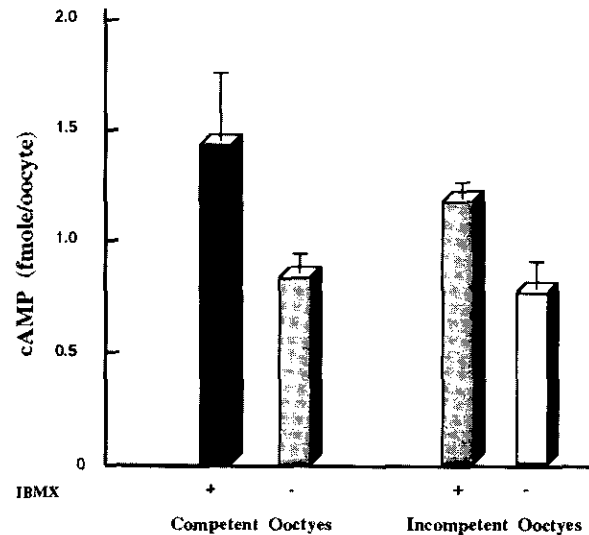

FIG. 4. cAMP content in incompetent rat oocytes. Either competent or incompetent oocytes were isolated into control medium in the presence or the absence of IBMX (0.2 mM). Concentrations of intraoocyte cAMP were determined at 2 hr of incubation by RIA in groups of 35–50 oocytes each. Error bars indicated SEM.

Concentrations of cAMP in fully grown mice and rat oocytes drop after their isolation from the ovarian follicles in association with reinitiation of meiosis (Schultz et al., 1983; Aberdam et al., 1987). Furthermore, spontaneous maturation in vitro in these oocytes is prevented when the relatively high intraoocyte concentrations of cAMP are maintained by phosphodiesterase inhibitors (Cho et al., 1974; Dekel and Beers, 1978; Dekel, 1987). A possible correlation between intracellular levels of cAMP and maintenance of meiotic arrest has been tested in growing oocytes. We found that similar to fully grown oocytes a drop in intraoocyte cAMP concentrations is observed upon the release of the growing oocytes from the ovarian follicles (Fig. 4). At 2 hr after isolation from the ovarian follicle into IBMX-free medium, the intracellular concentration of cAMP in growing oocytes was 0.77 ± 0.12 fmole/oocyte compared to 1.17 ± 0.07 fmole/oocyte determined in similar oocytes incubated in the presence of IBMX. The amount of cAMP in fully grown oocytes isolated into IBMX-containing medium (1.42 ± 0.3 fmole/oocyte) was apparently higher than that in growing oocytes incubated under similar conditions (1.17 ± 0.07 fmole/oocyte). However, considering the difference in their protein content (27.5 and 19.25 ng, respectively), the calculated concentrations of the intracellular cAMP are similar (0.052 and 0.061 fmole/ng protein, respectively).

DISCUSSION

Fully grown, but not growing, meiotically arrested mammalian oocytes undergo maturation in vitro spon-

taneously, upon their release from the ovarian follicles. Maturation *in vitro* can also be induced in follicle-enclosed fully grown rodent oocytes incubated with different hormones and nonhormonal agents. To test their ability to resume meiosis in response to hormonal stimulation, follicle-enclosed growing oocytes were incubated with either of the two gonadotropins, LH or FSH, as well as with a GnRH agonist analog and forskolin. With the exception of LH, all of these agents successfully induce maturation in fully grown rat oocytes that reside in both large and small antral follicles (Dekel *et al.*, 1983, 1988; Dekel and Sherizly, 1983). In the absence of receptors for LH on the granulosa cells at earlier stages of folliculogenesis (Richards and Midgley, 1976), the action of this gonadotropin has been shown by us previously to be limited to the large antral follicles (Dekel *et al.*, 1988). Since the growing oocytes reside in preantral follicles (Sorensen and Wasserman, 1976; Bar-Ami and Tsafriri, 1981), the failure of LH to stimulate resumption of meiosis in these oocytes was quite expected. However, receptors of either FSH or GnRH have been demonstrated in preantral rat follicles (Richards and Midgley, 1976; Smith and Ojeda, 1984). Furthermore, forskolin action in follicle-enclosed oocytes is independent of the presence of any specific receptors (Dekel *et al.*, 1988). It is, therefore, not the lack of the relevant receptors, but rather their incompetence to resume meiosis that prevented growing oocytes from responding to FSH, GnRHa, or forskolin. We suggest, therefore, that the term meiotic incompetence in mammals can be extended to include inability of the oocyte to mature not only spontaneously but also following hormonal triggering. Likewise, meiotic incompetence in *Xenopus* is defined as the failure of oocytes to resume meiosis in response to hormonal (progesterone) stimulation (Taylor and Smith, 1987).

Balakier (1978) was the first to demonstrate that fusion of maturing fully grown mouse oocytes caused GVB in incompetent growing oocytes and therefore has suggested that the incompetence to reinitiate meiosis might be due to the absence or the insufficient level of MPF. We hereby demonstrate for the first time that p34^{cdc2}, the catalytic subunit of MPF, is present in growing rat oocytes. Moreover, the concentration of this regulatory protein in growing and fully grown oocytes is apparently similar. The presence of p34^{cdc2} has also been demonstrated in growing *Xenopus* oocytes (Johnson and Smith, 1990). However, based on an increased intensity of the p34^{cdc2} band, as detected by immunoblot analysis, these authors suggest that this protein accumulates with oocyte growth. Nevertheless, during their growing process, oocytes increase significantly in size. Therefore, considering the different oocyte diameters, as presented in the above study, it seems that similar to the rat the

changes in the p34^{cdc2} protein concentrations in *Xenopus* oocytes during development are apparently negligible.

It has been well established that the mechanism of activation of MPF involves interaction between p34^{cdc2} and cyclin followed by dephosphorylation of the p34^{cdc2} kinase (reviewed by Norbury and Nurse, 1992). Therefore, the existence of p34^{cdc2} is essential but not sufficient for MPF activation. Any conclusion related to MPF activity in growing oocytes requires additional information with regard to the phosphorylation state of p34^{cdc2} and the expression of cyclin. Interestingly, growing mouse oocytes have been recently shown to express B1 and B2 cyclin mRNAs (Chapman and Wolgemuth, 1992, 1993). Cyclin B2 has also been found to be present and physically associated with a tyrosine phosphorylated p34^{cdc2} in small growing *Xenopus* oocytes (Rime *et al.*, 1991). However, it appears that the level of cyclin might be insufficient to activate the p34^{cdc2} kinase since it was only after microinjection of its mRNA that the growing *Xenopus* oocytes initiated meiosis (Johnson and Smith, 1990). Hence, cyclin that does not reach the threshold level necessary for MPF activation may be responsible for the state of meiotic incompetence in growing oocytes.

Differences in post-translational modifications of the protein components of MPF could possibly also account for meiotic incompetence. In this regard, a regulatory role for a type 2A phosphatase that inhibits a protein essential for MPF activation has recently been suggested (Felix *et al.*, 1990). This idea is supported by the demonstration that okadaic acid (OA), a specific inhibitor of protein phosphatases 1 and 2A (Choen *et al.*, 1990), successfully induced MPF activation and meiosis reinitiation in fully grown frog, starfish, and mouse oocytes (Goris *et al.*, 1989; Picard *et al.*, 1989; Rime and Ozon, 1990). Stimulation of GVB by OA has recently been demonstrated also in incompetent mouse oocytes (Gavin *et al.*, 1991). This report suggests that the activity of MPF in incompetent oocytes is also subjected to negative regulation by a type 2A phosphatase-sensitive protein.

Progressive changes in chromatin organization have been correlated with the expression of meiotic competence (McGaughey et al., 1979). Changes in chromatin organization appear to be coordinated with cytoplasmic microtubule alterations (Mattson and Albertini, 1990). It has recently been demonstrated that modifications in both chromatin and microtubule organization coincide with the acquisition of meiotic competence and are correlated with the ability to resume meiosis *in vivo* in mouse oocytes (Wirkramasinghe *et al.*, 1991). MAP kinases are known to phosphorylate microtubule associated protein 2. These kinases undergo phosphorylation and become active upon activation of MPF (Gotoh *et al.*, 1991). We hereby demonstrate the presence of two isoforms of MAP kinase in the incompetent rat oocytes. De-

tection of these enzymes at a similar quantity in growing and fully grown oocytes indicates that meiotic incompetence is apparently not due to a lack of expression of this cell cycle regulatory component.

The results discussed so far present similarities rather than differences between meiotically competent and incompetent oocytes. Even the levels of cAMP in fully grown oocytes, which seemed to be somewhat higher than those found in growing oocytes, appeared almost identical when normalized according to the intracellular protein content. Moreover, our observation that the decrease in intraoocyte cAMP levels is prevented by IBMX indicates that growing oocytes, like fully grown oocytes, possess an active cAMP phosphodiesterase (Bornslaeger et al., 1984). Furthermore, the drop in cAMP concentrations following isolation from the ovary suggests that incompetent oocytes, similar to the competent oocytes, exhibit a poor ability to generate cAMP. Since growing oocytes have been found to be metabolically coupled with the surrounding cumulus cells (Eppig, 1979; Piontkewitz and Dekel, unpublished), we further suggest that similar to fully grown oocytes, cAMP content in growing oocytes apparently originates from the neighboring follicular cells (reviewed by Dekel, 1988).

Our present study, however, does demonstrate a major difference between competent and incompetent oocytes with regard to the role of cAMP in regulation of meiotic arrest. Reinitiation of meiosis in fully grown oocytes is associated with a drop in intraoocyte cAMP that appears to be an initial step in a cascade of events eventually leading to oocyte maturation (reviewed by Dekel, 1988). One such event is apparently activation of MPF. Negative regulation of MPF in mammalian oocytes has recently been suggested. Specifically, it has been demonstrated in the mouse and further confirmed by us in the rat that maintenance of cAMP concentrations at a relatively high level not only inhibits oocyte maturation but also keeps the p34^{cdc2} in an inactive phosphorylated state (Choi et al., 1991; Goren and Dekel, 1994). In the present study we demonstrate, for the first time, that maintenance of meiotic arrest in incompetent oocytes is independent of intraoocyte cAMP concentrations. Therefore, MPF in incompetent oocytes does not seem to be subjected to negative regulation by this nucleotide.

Along with our line of evidence are previous reports that microinjection of the heat-stable inhibitor of cAMP-dependent protein kinase (PKI), which induces GVB in fully grown Xenopus and mouse oocytes, did not induce maturation in meiotically incompetent oocytes (Sadler and Maller, 1983; Bornslaeger et al., 1988). Moreover, the PKI microinjected into growing mouse oocytes did not allow the maturation associated changes in protein phosphorylation observed in the fully grown oocytes (Bornslaeger et al., 1988). These findings, in con-

junction with our present results, suggest that the cAMP-regulated step is not responsible for the meiotic arrest in mammalian growing oocytes and that the factor(s) responsible for meiotic incompetence might be located at a point that is distal to cAMP but prior to MPF along the cascade of biochemical regulators of oocyte maturation.

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