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Maintenance of Meiotic Arrest by a Phosphorylated $p34^{cdc2}$ Is Independent of Cyclic Adenosine 3′,5′-Monophosphate

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ABSTRACT

The meiotic division in oocytes is arrested in the G_2 phase of the cell cycle. Resumption of meiosis, also known as oocyte maturation, entails a G_2 to M transition and is associated with a drop in intraoocyte concentrations of cAMP. Recent studies imply that tyrosine dephosphorylation of $p34^{cdc2}$ is a prerequisite for entry into the M-phase of the cell cycle. Our study was designed to test the involvement of protein tyrosine phosphatase (PTPase)-regulated dephosphorylation of $p34^{cdc2}$ in resumption of meiosis in rat oocytes and to explore the possible control of this event by the intraoocyte concentrations of cAMP. Isolated rat oocytes undergoing meiotic maturation spontaneously in vitro served as our experimental model. We found that sodium metavanadate, an inhibitor of PTPase, reversibly blocked the spontaneous maturation in vitro of rat oocytes (ED_{50} = 0.26 mM). We further demonstrated that the vanadate-sensitive event is completed by 2 h after reinitiation of meiosis. Immunoblot analysis using specific antiphosphotyrosine antibodies revealed that vanadate caused accumulation of phosphotyrosine on a 34-kDa protein, also recognized by anti-$p34^{cdc2}$ antibodies. The phosphorylated form of $p34^{cdc2}$ was also detected in oocytes arrested in the G_2 phase by the phosphodiesterase inhibitor isobutyl methylxanthine (IBMX). Intraoocyte concentrations of cAMP in vanadate-inhibited oocytes were similar to those in oocytes that resumed meiosis spontaneously in vitro and lower than those in oocytes maintained in meiotic arrest by IBMX (0.073 ± 0.08, 0.84 ± 0.09, and 1.42 ± 0.3 fmol/oocyte, respectively). We conclude that a PTPase that regulates the phosphorylation state of $p34^{cdc2}$ participates in the control of meiosis in rat oocytes. Furthermore, maintenance of meiotic arrest by a phosphorylated $p34^{cdc2}$ that is independent of cAMP suggests that the above-mentioned PTPase activity occurs downstream to the oocyte maturation-associated drop of intracellular concentrations of cAMP.

INTRODUCTION

Protein tyrosine dephosphorylation has been implicated in the regulation of the exit from the G_2 phase and entry into the M-phase of the cell cycle (reviewed by Norbury and Nurse [1]). Investigations in somatic cells revealed that the catalytic subunit of the M-phase promoting factor (MPF), $p34^{cdc2}$ protein kinase, is maximally phosphorylated on tyrosine in the G_2 phase. The tyrosine-phosphorylated $p34^{cdc2}$ was found to be inactive, preventing the cells from entry into mitosis [2]. Tyrosine dephosphorylation of $p34^{cdc2}$ appeared to be one of a number of obligatory steps in the mitotic activation of this kinase leading to the G_2/M transition [2, 3]. Tyrosine and probably threonine in $p34^{cdc2}$ seem to be specifically dephosphorylated by the cdc25 gene product [4–6].

Meiosis is a particular example of cell division. The meiotic division in oocytes is a protracted process that is naturally arrested at the diplotene of the first prophase, which corresponds to the G_2 phase of the cell cycle. Meiotically arrested oocytes are referred to as immature oocytes. Resumption of meiosis in these oocytes is known as oocyte maturation and entails a G_2 to M transition. Therefore, immature oocytes represent a synchronous population of cells serving as a perfect system for investigating the G_2 control point of the cell cycle. Indeed, studies conducted on *Xenopus* and starfish oocytes have contributed significant information concerning the regulatory components that participate in the G_2/M transition. These studies revealed that oocytes arrested at the G_2 phase contain a $p34^{cdc2}$/cyclin B complex kept in an inactive form by phosphorylation of its $p34^{cdc2}$ subunit and that the transition into the M-phase involves $p34^{cdc2}$ dephosphorylation [7, 8]. A recent study on mammals revealed that in mouse oocytes $p34^{cdc2}$ undergoes changes in its phosphorylation state during meiotic maturation [9].

Mammalian oocytes can resume meiosis spontaneously in vitro upon their release from the ovarian follicles. The spontaneous maturation of mammalian oocytes is blocked by addition of membrane-permeable derivatives of cAMP or phosphodiesterase inhibitors to the incubation medium [10, 11]. Furthermore, a drop in intraoocyte concentrations of cAMP [12–14] followed by inactivation of the cAMP-dependent protein kinase (PKA [15]) is associated with reinitiation of meiosis. The biochemical events occurring downstream to the inactivation of PKA are not fully clarified. The study reported here was designed to test the involvement of a protein tyrosine phosphatase (PTPase) during resumption of meiosis in rat oocytes and to explore the possible regulation of such enzymatic activity by intraoocyte cAMP. Vanadate, an established inhibitor of PTPases [2, 16, 17], was employed in our experiments to prevent protein tyrosine dephosphorylation.

MATERIALS AND METHODS

Animals

Sexually immature Wistar female rats (23–24 days old) from our departmental colony received s.c. injections of 10

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IU of eCG (Intervet, Holland) in 0.1 ml of 0.9% NaCl. For collection of preovulatory oocytes, rats were killed by cervical dislocation 48 h after eCG administration. For collection of postovulatory oocytes, rats received further injections (i.p.) of 5 IU of hCG (Pregnyl, Organon, Oss, Holland) 52 h after administration of eCG and were killed 20 h after hCG injection.

Culture of Cumulus-Enclosed Oocytes

Ovaries were removed and placed in Leibovitz's L-15 tissue culture medium (Gibco, Grand Island, NY) containing penicillin (100 IU/ml, Gibco) and streptomycin (100 μg/ml, Gibco). This composition is referred to as control medium. Cumulus-oocyte complexes were released from the large antral follicles into the medium by use of iris forceps [11]. The cumulus-oocyte complexes were placed in a 200-μl drop of control medium containing the indicated concentrations of sodium metavanadate ($NaVO_3$; BDH Chemicals Ltd., Poole, UK) in 35-mm Petri dishes for various times of incubation at 37°C in air at a relative humidity of 100%. Recovery from the effect of vanadate was studied in cumulus-oocyte complexes washed three times and further incubated in vanadate-free control medium. At the end of incubation, the oocytes were analyzed for maturation by Nomarski interference contrast microscopy. If the germinal vesicle (GV) was present, oocytes were classified as meiotically arrested. Resumption of meiosis was indicated by the absence of the GV (including the nucleus) in the individual oocytes (GV breakdown, GVB).

Isolation of Cumulus-Free Oocytes

Preovulatory oocytes. Preovulatory cumulus-free oocytes were obtained as described previously [18]. Briefly, cumulus-oocyte complexes recovered from the ovarian follicles as described above underwent several passages through a glass micropipette (approx. 100 μm in diameter) after a 30-min incubation at 37°C in control medium containing collagenase (50 IU/ml; Sigma, St. Louis, MO) and EDTA (2.0 mM, Sigma) at pH 7.2. The resulting cumulus-free oocytes were washed four times and transferred into polypropylene test tubes in a minimum volume of medium.

Postovulatory oocytes. Postovulatory oocytes were recovered from the oviductal ampullae. For this purpose, oviducts were isolated into control medium containing hyaluronidase (1 mg/ml, Sigma), and the cumulus-oocyte complexes were released into the medium. After 20 min at room temperature, the cumulus cells were mechanically removed, and the oocytes collected as described above.

Determination of cAMP in the Oocytes

Intraoocyte cAMP was determined by cAMP ^{125}I RIA [19]. After incubation with or without the indicated agents, cumulus-free oocytes were washed four times and transferred with a minimal volume of control medium into sodium acetate buffer (50 mM, pH 6.5), containing 0.2 mM of 3-isobutyl-1-methylxanthine (IBMX, Sigma). The oocytes were then collected in 5 μl of buffer and transferred into polypropylene test tubes. Repeated freezing and thawing (three times) of the oocytes on liquid nitrogen was used to break the membranes and release the cAMP content into the buffer. A similar volume of medium of the last wash was identically treated to obtain blank values for intraoocyte cAMP determinations. The samples were kept at −80°C. For cAMP determination, each sample was lyophilized and reconstituted with 300 μl of sodium acetate buffer (50 mM, pH 6.5). Each sample contained 70–100 oocytes, and its cAMP content was tested in duplicate. The sensitivity range of the assay was 10–80 fmol/sample. An aliquot of a pooled sample of oocytes was included in each individual assay for normalization of interassay variations.

Antibodies

Polyclonal rabbit anti-phosphotyrosine antibodies were purchased from Zymed (San Francisco, CA); antisera raised in rabbits against the PSTAIR peptide of p34^{cdc2} was kindly provided by Prof. Y. Yarden, The Weizmann Institute of Science, Rehovot, Israel.

Western Blotting

Cumulus-free oocytes were transferred to polypropylene test tubes and centrifuged at 1000 rpm for 2 min. The supernatant was removed, and the pellet was resuspended in lysis buffer (10 oocytes in 1.0 μl of 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 [20]). The lysates were vortexed and incubated on ice for 5 min and then centrifuged for 5 min at 14 000 × g. The supernatants were frozen on liquid nitrogen and kept at −80°C. Before electrophoresis, the extracts of each sample were mixed with concentrated (triple-strength) electrophoresis sample buffer (5 μl/100 oocytes) and heated to 90°C for 5 min.

For detection of the p34^{cdc2} kinase, proteins were separated on 12.6% SDS-polyacrylamide gels by use of a 13-cm gel apparatus (Hoefer, San Francisco, CA). Separation of proteins by 7.5%-15% SDS-polyacrylamide gradient gels served to detect phosphotyrosine on the entire repertoire of cellular proteins (13-cm gel apparatus, Hoefer). At the end of electrophoresis, the separated proteins were transferred to a nitrocellulose membrane, (Hybond-C super; Amersham, Arlington Heights, IL) by means of a Milliblot Trans-blot apparatus (Bio-Rad, Richmond, CA) for 16 h at 100 mA in transfer buffer (33 mM Trizma base, 192 mM glycine, 20% methanol). The membranes were then stained with Ponceau for comparison of protein amounts transferred in the different lanes. For anti-phosphotyrosine and anti-PSTAIR immunoblot analysis, membranes were blocked

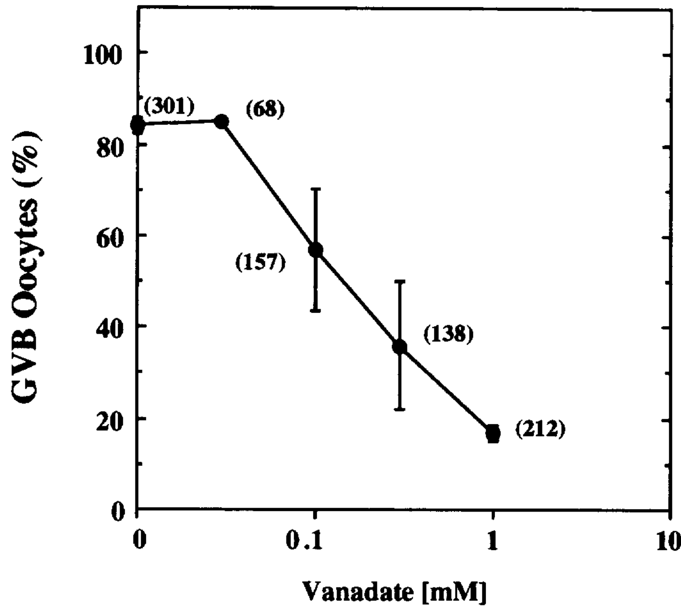

FIG. 1. Dose response of inhibitory effect of vanadate on spontaneous maturation of isolated rat oocytes. Cumulus-enclosed oocytes were incubated in the presence or absence of indicated concentrations of vanadate. Oocytes were examined for the presence or absence of GV after 4 h in culture. Means ± SEM of four individual experiments are presented. Total number of oocytes examined is indicated.

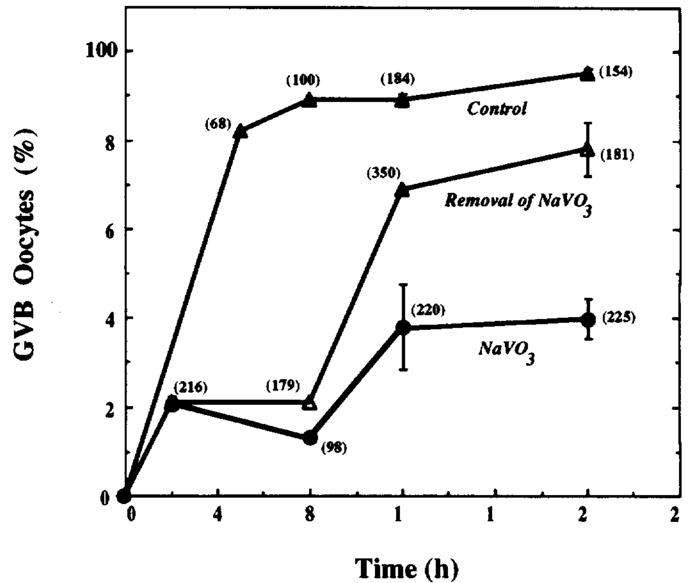

FIG. 2. Reversibility of inhibitory effect of vanadate on spontaneous maturation of isolated rat oocytes. Cumulus-enclosed oocytes were incubated in the presence or absence of vanadate (0.5 mM). After 2 h of incubation a subpopulation of the oocytes incubated with vanadate was transferred to vanadate-free medium. The fraction of oocytes containing GV was examined after indicated periods of incubation. Means of five individual experiments are presented. Total number of oocytes examined is indicated.

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 h at 4°C. The membranes were then incubated with anti-phosphotyrosine (1:2000) or anti-PSTAIR (1:500) overnight at 4°C and washed three times (30 min each wash) in TTBS. The blots were then probed with the enhanced chemiluminescence (ECL) Western blotting detection system (Amersham) and autoradiographed.

RESULTS

To test the possibility that a PTPase-regulated tyrosine dephosphorylation is essential for reinitiation of meiosis, immature, G_2-arrested oocytes were isolated into culture medium containing sodium metavanadate, an established inhibitor of PTPases [16, 17]. We found that vanadate effectively inhibited the spontaneous maturation of rat oocytes. Vanadate action was dose-dependent with an apparent ED_{50} of 0.26 mM (Fig. 1). The inhibitory effect of vanadate was reversible, with 70% of the oocytes resuming meiosis at 10 h after removal of this agent from the culture medium (Fig. 2).

To analyze the temporal relationships between the onset of meiosis and the vanadate-sensitive event, we transiently exposed isolated oocytes to vanadate-free medium and then added this inhibitory agent at different time points of incubation. Those oocytes that underwent irreversible commitment to resume meiosis, exhibiting insensitivity to the inhibitory action of vanadate, could be detected only after a total 4 h of incubation that allowed them to lose their GV.

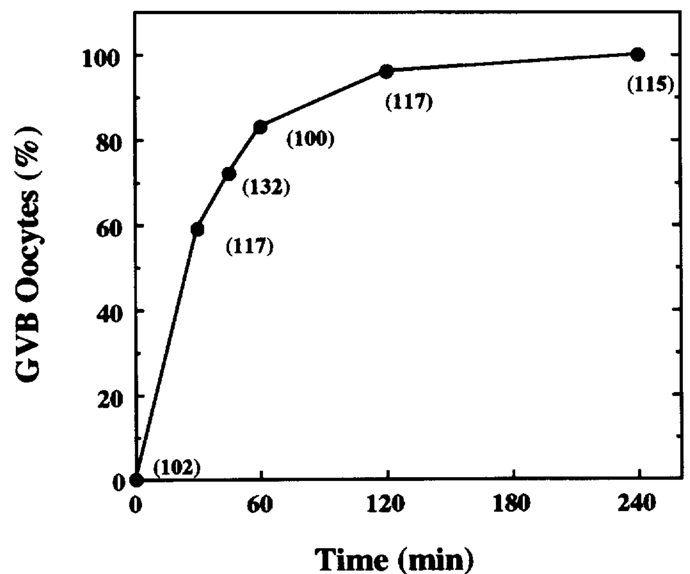

FIG. 3. Timing of the vanadate-inhibited step during spontaneous maturation of isolated rat oocytes. Cumulus-enclosed oocytes were isolated into inhibitor-free (control) medium. Vanadate (0.5 mM) was added to the medium at indicated time-points. Individual oocytes were examined for the presence or absence of GV after a total of 4 h in culture. Means of two individual experiments are presented. Total number of oocytes examined is indicated.

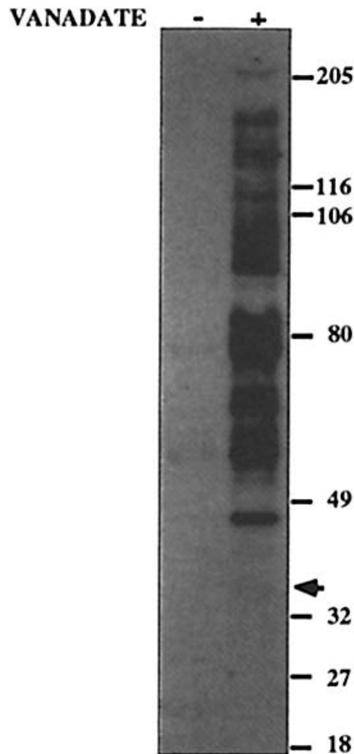

FIG. 4. Protein tyrosine phosphorylation pattern of immature, vanadate-arrested, and spontaneously maturing rat oocytes. Preovulatory cumulus-free oocytes were isolated into culture medium in the presence or absence of vanadate (0.5 mM). After 2 h oocytes were lysed. Samples of 300 oocytes per lane were separated by electrophoresis on a 7.5%-15% acrylamide gradient gel. Proteins were then immunoblotted with anti-phosphotyrosine antibodies (1:2000) followed by ECL detection. The resulting autoradiogram is shown. The experiment was performed three times and similar results were obtained in each case. Shown is a representative example.

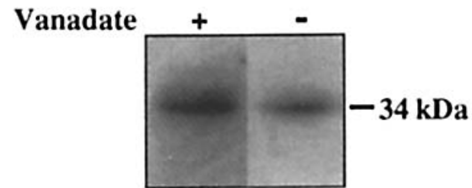

FIG. 5. Immunoblot analysis of p34^{cdc2} in immature, vanadate-arrested, and spontaneously maturing rat oocytes. Lysates of cumulus-free oocytes (300 oocytes/lane) were separated by electrophoresis on a 12.6% acrylamide gel. Proteins were then immunoblotted with anti-PSTAIR antibodies (1:500) followed by ECL detection. The resulting autoradiogram is shown. The experiment was performed three times and similar results were obtained in each case. Shown is a representative example.

These experiments revealed that vanadate added at 120 min after isolation of the oocytes from the ovarian follicle could no longer inhibit the resumption of meiosis (Fig. 3). These findings suggest that the vanadate-sensitive step takes place at 2 h after the onset of meiosis, that is, about 2 h before the actual disappearance of the GV.

Immunoblot analysis using specific anti-phosphotyrosine antibodies was further performed on isolated rat oocytes undergoing meiotic maturation spontaneously as compared to oocytes maintained in meiotic arrest by vanadate. We found that exposure to vanadate caused a massive accumulation of phosphotyrosines on several polypeptides, among which a 34-kDa protein could be detected (Fig. 4).

To investigate the possibility that this 34-kDa phosphotyrosyl protein represents p34^{cdc2}, extracts of rat oocytes were subjected to immunoblot analysis using specific antibodies directed against the PSTAIR peptide representing the cdc2 protein [21]. We found that in G_2 oocytes meiotically arrested by vanadate, the anti-p34^{cdc2} antibodies recognized two bands with slightly different electrophoretic mobility (Fig. 5). The upper band, which was also recognized by antiphosphotyrosine antibodies in oocytes subjected to gel electrophoresis in a parallel lane, apparently represents the phosphorylated form of the protein. The antiphosphotyrosine antibodies did not recognize the lower band, suggesting that this form represents nonphosphorylated p34^{cdc2}.

The two forms of p34^{cdc2} were also detected by anti-p34^{cdc2} antibodies in oocytes maintained in meiotic arrest by IBMX (Fig. 6). The upper, phosphorylated form of p34^{cdc2} could still be observed in oocytes at 30 min after their transfer into IBMX-free medium (data not shown), but disappeared within 2 h of incubation under these conditions (Fig. 6). The nonphosphorylated p34^{cdc2} was present throughout the entire meiotic cell cycle and was also detected in postovulatory oocytes arrested at Metaphase II (Fig. 6).

As stated in the *Introduction*, maturation of rat oocytes is negatively regulated by intracellular concentrations of cAMP. We further investigated the possible interrelationships between intraoocyte cAMP and protein tyrosine dephosphorylation. Our experiments confirmed earlier findings [14] that intracellular concentrations of cAMP in G_2, IBMX-arrested rat oocytes are maintained at relatively high concentrations and that at 2 h after isolation from the ovarian follicles into IBMX-free medium, intraoocyte concentrations of cAMP drop significantly. We further demonstrated that a drop in intraoocyte concentrations of cAMP

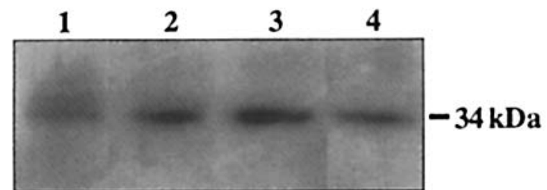

FIG. 6. Immunoblot analysis of p34^{cdc2} in immature, IBMX-arrested, spontaneously maturing, and postovulatory rat oocytes. Lysates of cumulus-free oocytes (300 oocytes/lane) were separated by electrophoresis on a 12.6% acrylamide gel. Proteins were then immunoblotted with anti-PSTAIR antibodies (1:500) followed by ECL detection. The resulting autoradiogram is shown. Lane 1: preovulatory, immature oocytes incubated for 2 h with 0.2 mM IBMX; lanes 2 and 3: preovulatory, mature oocytes incubated for 2 h and 10 h, respectively, in IBMX-free medium; lane 4: postovulatory, mature oocytes. The experiment was performed twice, and similar results were obtained in each case. Shown are the results of one experiment.

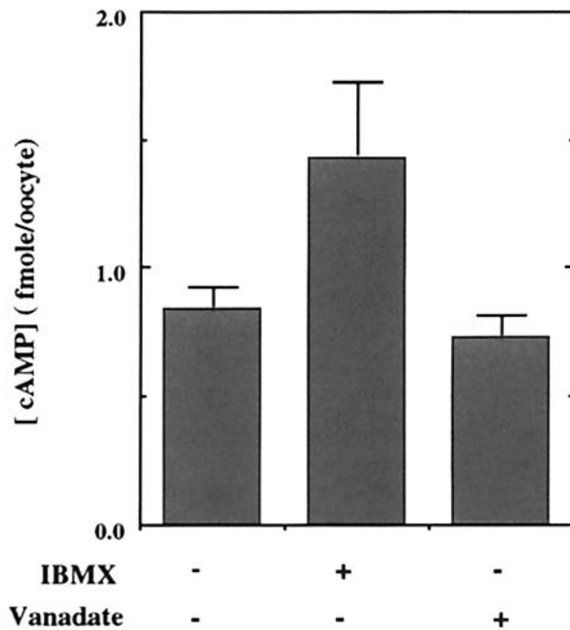

FIG. 7. Cyclic AMP concentrations in spontaneously maturing and either IBMX or vanadate-inhibited rat oocytes. Cumulus-free oocytes were isolated into culture medium in the presence or absence of either IBMX (0.2 mM), or vanadate (0.5 mM). Concentrations of intraoocyte cAMP were determined at 2 h of incubation by RIA. Mean ± SEM of five individual experiments is presented.

is also obtained in the vanadate-incubated oocytes (Fig. 7) in spite of its inhibitory effect on resumption of meiosis.

DISCUSSION

Our present study demonstrates that 1) vanadate, an inhibitor of PTPase, reversibly blocks the resumption of meiosis in isolated rat oocytes, 2) vanadate-treated oocytes contain a tyrosine-phosphorylated 34-kDa protein that is recognized by anti-$p34^{cdc2}$ antibodies, 3) a phosphorylated $p34^{cdc2}$ is also present in IBMX-treated oocytes, and 4) vanadate inhibition of oocyte maturation is independent of cAMP.

Dephosphorylation of $p34^{cdc2}$ during the early phase of the first meiosis that is inhibited by vanadate has been previously shown in murine oocytes [9, 22]. Using rat oocytes, we demonstrated in the study reported here that vanadate-inhibited dephosphorylation on a tyrosine residue of $p34^{cdc2}$ is associated with maintenance of oocytes in a state of meiotic arrest. Taken together, these results suggest that a PTPase activity participates in the regulation of meiosis in mammalian oocytes.

Vanadate inhibition of PTPase has been previously employed to demonstrate the essential role of $p34^{cdc2}$ tyrosine dephosphorylation during the G_2/M transition of the mitotic cell cycle [2]. The effective dosage used in this study (50 μM) is within the range of concentrations employed in our experiments to prevent rat oocytes from entry into the M-phase of meiosis. Indeed, vanadate is known as a potent and selective inhibitor of PTPases when used in the micromolar range in cell-free systems [1, 16, 17]. Therefore, the stimulation of *Xenopus* oocytes to resume meiosis obtained by 5–10 mM of vanadate reported by Hainaut et al. [23] probably involved a set of biochemical reactions other than selective inhibition of PTPase activity.

Several reports have suggested that the cdc25 protein product that stimulates the removal of inhibitory phosphate residues on $p34^{cdc2}$ is a highly conserved positive regulator of the M-phase. It has been shown in *Xenopus* oocytes that the cdc25 PTPase can directly dephosphorylate the Tyr^{15} residue of $p34^{cdc2}$ and that injection of this cdc25 protein into *Xenopus* oocytes activated the cyclin/$p34^{cdc2}$ complex and induced the G_2/M transition [4]. A more recent study has demonstrated that the phosphatase activity of the cdc25 protein changes in a cell cycle-dependent manner, being controlled by phosphorylation/dephosphorylation of this enzyme [24]. Similar to previous findings in the mouse [9], our findings here demonstrate that dephosphorylation of $p34^{cdc2}$ is inhibited by IBMX, which maintains the intraoocyte concentration of cAMP at a relatively high level. These findings offer circumstantial evidence for a possible correlation between the decrease in cAMP and $p34^{cdc2}$ dephosphorylation in the control of meiosis. They may further suggest that cAMP could elicit its negative regulatory action on oocyte maturation by activation of a series of biochemical events that finally lead to inhibition of either the cdc25 protein or another, still unidentified PTPase activity that might be necessary for resumption of meiosis.

Negative regulation of meiosis by cAMP was originally proposed by Cho and his colleagues [10]. These investigators demonstrated that spontaneous maturation in vitro of mouse oocytes released from their ovarian follicles can be reversibly blocked by addition of a membrane-permeable derivative of cAMP or a phosphodiesterase inhibitor. These findings were later extended to include other mammalian species (rat [11]; pig [25]; cow [26, 27]) and led to the hypothesis that this cyclic nucleotide could serve as the physiological inhibitor involved in the maintenance of meiotic arrest. This hypothesis was supported by a number of lines of evidence. First, cAMP derivatives and inhibitors of phosphodiesterase prevent not only spontaneous but also LH-stimulated maturation in follicle-enclosed oocytes in vitro [28, 29]. Second, determinations of the intracellular levels of cAMP in rat and mouse oocytes revealed that postovulatory mature oocytes contain lower levels of cAMP than follicular immature oocytes [12, 30]. Moreover, such experiments also demonstrated that spontaneous maturation in vitro is preceded by a sharp drop in intraoocyte cAMP, while no decrease in cAMP concentrations is observed in oocytes maintained in meiotic arrest by a phosphodiesterase inhibitor [14, 31]. Clear correlation between the meiotic status and intraoocyte levels of cAMP was also demonstrated in nonmammalian species (amphibia [32]; fish [33]; starfish [34]), implicating a universal role for cAMP as a negative regulator of oocyte maturation. Furthermore, an inverse cor-

relation between cAMP levels and proliferation, which has been described for certain somatic cell types in culture [35], may imply that cAMP plays a regulatory role not only in the meiotic but also in the mitotic cell cycle. Along with this line of evidence, down-regulation of a cAMP-dependent protein kinase (PKA) that is essential for the induction of mitosis in mammalian somatic cells has been recently suggested [36].

The central role of cAMP in the control of meiosis is well established. Furthermore, it is experimentally evident that the negative action of cAMP on resumption of meiosis is mediated by an activated PKA in both mammalian and amphibian oocytes [15, 32]. However, the biochemical events occurring downstream to cAMP activation of PKA are as yet not fully clarified. Classical fusion experiments, performed soon after the discovery of the central role of MPF in regulation of meiosis, proposed that MPF activation is inhibited by cAMP. These studies demonstrated that *Xenopus* oocytes are induced to resume meiosis after fusion with maturing, but not with dibutyryl cAMP-arrested, mouse oocytes [37]. On the other hand, fusion of metaphase II mouse oocytes rapidly induced GVB in dibutyryl cAMP-arrested homologous oocytes, suggesting that once cytoplasmic MPF has been activated, its ability to induce the transition into M-phase is no longer sensitive to cAMP [38]. Recent studies in *Xenopus* further demonstrated that cAMP inhibits the activation of p34^{cdc2} kinase, the catalytic subunit of MPF, that this action of cAMP is reversed by microinjection of an inhibitor of PKA [39], and that the PKA regulatory action is mediated by the *mos* protooncogene product [40]. In mammalian oocytes, on the other hand, experimental evidence related to the biochemical events that link the cAMP-activated PKA to the regulatory elements of the cell cycle is not yet available. One such link suggested by our present study is a PTPase activity that could be possibly regulated (either directly or indirectly) by PKA.

The possible participation of a PTPase in regulation of meiosis in mammals has been investigated in our present study by the use of vanadate for experimental manipulation of protein tyrosine phosphorylation/dephosphorylation, as correlated to alternation between meiotic arrest and resumption of meiosis. Furthermore, to explore the possible regulation of such enzymatic activity by cAMP, we analyzed the effect of vanadate on the state of phosphorylation of p34^{cdc2} in combination with determinations of intraoocyte concentrations of this nucleotide. The results of our experiments reveal for the first time that a phosphorylated p34^{cdc2} maintains rat oocytes in meiotic arrest even under conditions of relatively low concentrations of cAMP. Our results may suggest that the decrease in intraoocyte cAMP and dephosphorylation of p34^{cdc2} represent parallel pathways, each of which is necessary but not sufficient for progression through meiosis. Alternatively, the maturation-associated drop in intraoocyte concentrations of cAMP may occur upstream and possibly lead to tyrosine dephosphorylations of p34^{cdc2}. Taking into account that in mammalian ([9]; our present study), as well as amphibian oocytes [39] cAMP inhibits dephosphorylation and activation of p34^{cdc2}, the second option for interpretation of our results seems more likely.

Taken together, our present results with rats and the previously reported observations in mice [9] are compatible with the following sequence of events. Either hormonal stimulation or, alternatively, separation of the mammalian oocyte from the ovarian follicle results in a decrease in intraoocyte concentrations of cAMP, which in turn leads to inactivation of PKA. Through an as yet unknown cascade of biochemical events, the down-regulated PKA allows activation of a PTPase that dephosphorylates the p34^{cdc2}, rendering it active. The activated p34^{cdc2} sets into motion the cellular machinery involved in resumption of meiosis.

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