Transient Exposure of Rhesus Macaque Oocytes to Calyculin-A and Okadaic Acid Stimulates Germinal Vesicle Breakdown Permitting Subsequent Development and Fertilization

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ABSTRACT

Exposure of mammalian oocytes to the protein phosphatase (PP)-1 (PP1) and PP2A inhibitor okadaic acid (OA) stimulates oocyte meiosis. However, treated oocytes do not develop beyond metaphase I (MI), and they display morphological abnormalities. Experiments were conducted to define inhibitor treatment conditions for macaque oocytes that would result in germinal vesicle breakdown (GVB) stimulation and completion of meiosis without significant cytoplasmic abnormalities. As described above for OA, continual exposure of macaque oocytes to 50 nM calyculin-a (CL-A) significantly enhanced GVB at 24 h compared to that in controls, and the majority of the treated oocytes displayed cytoplasmic abnormalities. However, transient exposure (10 min) of rhesus macaque oocytes to either 50 nM CL-A or 1.0 µM OA enhanced GVB rates compared to that in controls and did not increase the incidence of cytoplasmic abnormalities. Meiotic maturation from germinal vesicle-intact oocytes to MII was enhanced following transient treatment with CL-A or OA compared to that in controls; however, development from MI to MII occurred at a similar frequency. In vitro-matured oocytes transiently exposed to OA and CL-A were capable of fertilization. In addition, ovarian immunohistochemical analysis revealed that both PP1 and PP2A were present in macaque oocytes. PP1 was localized throughout the cytoplasm with a predominance in the nucleus, whereas PP2A was evenly distributed throughout the cytoplasm with a reduction in the nuclear area. These results taken together—differential developmental responses to inhibitor treatment and intracellular enzyme localization—may be indicative of multiple regulatory roles of PP1 and/or PP2A during meiosis.

INTRODUCTION

At the time of birth, mitotically proliferative mammalian oocytes enter prophase of meiosis I and remain in this arrested state until puberty, when hormonal stimulation recruits a subpopulation of oocytes and initiates their growth as well as the development of their surrounding follicular cells. In the periovulatory period, the gonadotropin surge triggers the oocyte to reinitiate meiosis leading to the maturation of the oocyte through meiosis I, extrusion of the first polar body, and subsequent arrest at metaphase of meiosis II just prior to ovulation [1]. Classical research by Pin-

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While the stimulatory influence of OA on Xenopus [13], starfish [14], bovine [15], and mouse [16–19] oocyte GVB has been reported, OA-enhanced GVB was accompanied by cytoplasmic abnormalities and a block in progression to metaphase (M) II. All of these studies used extended (2–16 h) exposure to or microinjection of relatively high con-
concentrations of OA. As stated in each of these reports, OA inhibits the activity of both PP1 and PP2A, and at present there is no direct identification of PP1 or PP2A in mammalian oocytes.

Our purpose was to determine whether variations in PP pharmacological inhibitor treatments of macaque oocytes would stimulate GVB without subsequent cytoplasmic abnormalities and allow development to MII. In addition, we have addressed the question of which PP is present in the macaque oocyte, PP1 and/or PP2A.

MATERIALS AND METHODS

Ovarian and Oocyte Collection

Adult female rhesus macaques were housed and cared for at the Oregon Regional Primate Research Center under NIH animal care and use guidelines. Ovaries were collected by paramedian pelvic laparotomy independent of menstrual cycle stage. Ovaries were placed into a modified Tyrode’s medium (TALP-Hepes + 0.3% BSA (fraction V; Sigma Chemical Co., St. Louis, MO)) [20] and transported to the laboratory, where they were bisected sagittally and either fixed in freshly prepared 4% paraformaldehyde, rinsed in PBS, and embedded in paraffin or used for isolation of germinal vesicle-intact (GVI) oocytes. Under a dissecting microscope, oocyte isolation was performed in TALP-Hepes + 0.3% BSA; individual antral follicles (≥500 μm in diameter) were ruptured with a 25-gauge needle, and the expelled oocyte-cumulus complexes were transferred into TALP-Hepes + 0.3% BSA containing 300 μg/ml hyaluronidase. After hyaluronidase exposure, the majority of cumulus cells were dispersed by aspiration into a narrow-bore pasteur pipette. Only oocytes with an intact germinal vesicle, with vitelline diameter greater than 100 μm, and without degeneration signs (vacuoles) that had previously been enclosed by more than two layers of cumulus cells were used in this study.

Oocyte In Vitro Maturation

In the first experiment, oocytes from two animals were randomly allocated to 50-μl drops (1 oocyte per drop) of TALP + 0.3% BSA alone (control) or containing 50 nM CL-A. Microdrops were overlaid with TALP-equilibrated mineral oil (Sigma) and incubated at 37°C in a humidified 5% CO₂ atmosphere for 24 h. Oocytes were evaluated, in a blinded fashion with regard to treatment conditions, using an inverted microscope with Hoffman optics (x400) at the time they were placed into culture and 24 h later. Appearance of the germinal vesicle was monitored as GVB, and evidence of cytoplasmic abnormalities included organelle withdrawal, uneven cytoplasm, and cytoplasmic fragmentation. In a second experiment, oocytes from 5 macaques were divided randomly between treatments that consisted of a 10-min incubation in TALP-Hepes + 0.3% BSA either alone (control) or containing 50 nM CL-A or 1.0 μM OA. After these transient treatments, oocytes were washed through five changes of TALP-Hepes + 0.3% BSA (1 ml) to remove residual PP inhibitor. Individual oocytes were then placed into 50-μl drops of TALP + 0.3% BSA, overlaid with TALP-equilibrated mineral oil, and incubated at 37°C in a humidified 5% CO₂ atmosphere for 48 h. Oocytes were assessed for nuclear maturation and cytoplasmic aberrations as described above at the time they were placed in culture and 1, 24, and 48 h later.

Semen Preparation and In Vitro Fertilization

Semen was collected by penile electroejaculation and processed for insemination as described previously [21]. Oocytes that had completed maturation to MII within 48 h of culture were transferred individually into 500 μl of TALP + 0.3% BSA under oil and inseminated with 200 000 activated/capacitated sperm. At 16 h after insemination, oocytes were examined for evidence of fertilization as indicated by the presence of two polar bodies and two pronuclei. Fertilized oocytes were cultured in vitro for evidence of timely cleavage prior to cryopreservation [22].

Immunohistochemistry

Fixed ovarian tissue was embedded in paraffin, and 5-μm sections were placed on superfrost-plus slides (Fisher Scientific, Itasca, IL), deparaffinized, placed in 100 mM glycine buffer (pH 3.65), and microwaved for 10 min for antigen retrieval. Slides were then blocked by placement in Tris-buffered saline (TBS) containing 5.0% dimethyl sulfoxide and 0.2% Tween 20 for 10 min, rinsed in TBS, and incubated for 10 min in TBS containing 0.3% BSA, 1 mg/ml sodium azide, and 1.6% normal goat serum (Vector Laboratories, Burlingame, CA). In addition, endogenous avidin and biotin were blocked with an avidin/biotin blocking kit (Vector). Slides were rinsed and incubated overnight with the appropriate primary antibody at room temperature in a humidified chamber; slides were then rinsed, washed (TBS + 0.05% Tween 20), rinsed again, and incubated with the appropriate biotinylated secondary antibody for 30 min at room temperature. After secondary antibody exposure, slides were quenched in 3.0% H₂O₂ in 90% methanol for 30 min, then rinsed and incubated for 30 min in avidin-biotin conjugated to peroxidase (Vector). After several rinses, sections were exposed to 0.025% 3,3’-diaminobenzidine (Dojindo Labs-Wako Chemical, Richmond, VA), rinsed, counterstained with Mayer’s hematoxylin (Sigma), dehydrated by three changes of ethanol and then three changes of xylene, and mounted. Positive controls consisted of mouse liver (not shown) and heart incubated with various antibodies. Negative controls included 1) elimination of the primary antibody, 2) nonimmune rabbit serum in place of the primary antibody, and 3) antibody preabsorption with respective antigenic peptides prior to utilization in immunohistochemistry.

Statistical Analysis

Data collected with respect to percentage of oocytes that underwent GVB, development to MII, and development of cytoplasmic abnormalities were compared within experiments (continual and transient PP inhibitor) between treatment groups within individual time points by way of z-test of proportions. Differences were considered to be significant when p < 0.05.

RESULTS

Continual CL-A Treatment and Oocyte Meiosis

Continuous exposure of monkey GVI oocytes to the PP1/PP2A inhibitor CL-A (50 nM) for 24 h significantly enhanced the percentage of oocytes that resumed meiosis (78% GVB) compared to that for controls (23% GVB; p < 0.001; Table 1). Similar to what was seen in the mouse studies mentioned above with use of OA, the prolonged exposure of monkey GVI oocytes to 50 nM CL-A resulted
in a higher percentage of oocytes displaying cytoplasmic abnormalities (83%) in comparison to control cultures (8%; \( p < 0.001 \)). These cytoplasmic aberrations consisted of grainy cytoplasm, oolemma "ruffling," vacuoles, organelle withdrawal, and cytoplasmic fragmentation, alone or in combination (Fig. 1). It appears that inhibition of PP1 and/or PP2A with CL-A enhances GVB but that continuous exposure is detrimental to oocyte cytoplasmic integrity and normal meiotic progression.

**Transient CL-A and OA Exposure and Oocyte Meiosis**

Cytoplasmic aberrations have been reported following continual, but not transient, treatment of hepatocytes with OA [23, 24]. Therefore, the influence of short-term exposure of macaque GVI oocytes to CL-A and OA was examined. Because prolonged CL-A treatment significantly enhanced the percentage GVB of oocytes at 24 h, an earlier time point following treatment (1 h) was also included. Transient exposure (10 min) of macaque GVI oocytes in TALP-Hepes alone, or in the presence of 50 nM CL-A or 1.0 \( \mu \)M OA followed by extensive washing

<table>
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<th>Treatment</th>
<th>n</th>
<th>GVB</th>
<th>Cytoplasmic aberrations</th>
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<tr>
<td>TALP (control)</td>
<td>13</td>
<td>23a</td>
<td>8a</td>
</tr>
<tr>
<td>TALP + 50 nM CL-A</td>
<td>18</td>
<td>78b</td>
<td>83b</td>
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*ab For each row, values with different alphabetic superscripts are significantly different (\( p < 0.001 \)).

FIG. 1. Photomicrograph of morphologically aberrant macaque oocytes following 24-h continual treatment in TALP containing 50 nM CL-A. GVI oocytes, with a perivitelline diameter \( \geq 100 \mu \)m, were collected from antral follicles and cultured for 24 h in the presence or absence of 50 nM CL-A. At 24 h, oocytes were microscopically observed for GVB and signs of abnormal morphology such as A) oolemma ruffling, B) vacuole formation, C) organelle withdrawal, or D) cytoplasmic fragmentation. Hoffman optics, \( \times 400 \) optical magnification (reproduced at 73%).

FIG. 2. GVB of rhesus macaque oocytes following transient exposure to the PP1/PP2A inhibitors CL-A and OA. GVI oocytes, with a perivitelline diameter \( \geq 100 \mu \)m, were collected from antral follicles and exposed for 10 min to control medium (TALP-Hepes) alone or containing 50 nM CL-A or 1.0 \( \mu \)M OA prior to culture. Oocytes were microscopically assessed for signs of GVB at 1, 24, and 48 h of culture. Comparisons were made only between treatment groups within individual time points. For each time in culture, bars with different letters are significantly different (\( p < 0.05 \)).

FIG. 3. Representative rhesus macaque oocytes and a 2-cell embryo following transient exposure to OA. GVI (arrowhead) oocytes (A) were collected from antral follicles, exposed to either 50 nM CL-A or 1.0 \( \mu \)M OA for 10 min, washed, and then placed into culture. Oocytes were microscopically assessed for GVB (B) and extrusion of the first polar body (development to MII; C). Those oocytes that had matured to MII by 48 h were inseminated with 200,000 sperm and assessed for fertilization and signs of early embryonic development (D). Hoffman optics, \( \times 400 \) optical magnification (reproduced at 68%).
and 1-h culture, resulted in a significant enhancement of GVB in those oocytes treated with the PP inhibitors (control, 17%; CL-A, 48%; OA, 44%; Fig. 2). The percentage of oocytes undergoing GVB following transient CL-A treatment was increased at both 24 h (67%) and 48 h (67%) compared to the values in controls (47% and 50%, respectively); however, this increase was not significant. Transient OA treatment significantly increased the percentage of oocyte GVB at 24 and 48 h (78% and 88%, respectively) compared to that in controls. In addition, short-term exposure of oocytes to CL-A resulted in only 7% of the oocytes displaying cytoplasmic abnormalities by 24 h of culture, a value that remained constant during the following 24 h. Transient OA treatment resulted in 3% and 6% of oocytes containing cytoplasmic abnormalities at 24 and 48 h, respectively (Table 2). Therefore, transient inhibition of PP1 and/or PP2A increased both the rate of meiotic resumption and the percentage of oocytes undergoing GVB, yet did not lead to increased cytoplasmic abnormalities.

After enhancing the percentage of oocytes undergoing GVB, transient PP inhibitor treatments did not interfere with meiotic progression to MII (Fig. 3C). There was no significant difference in the proportion of oocytes developing from GVI or MI to MII at 24 or 48 h of culture between control, CL-A, and OA treatments. The overall MII yield from GVI at 48 h of culture was 17%, 26%, and 38% in oocytes exposed to control, CL-A, or OA, respectively. By 48 h of culture, 33%, 39%, and 43% of oocytes that had undergone GVB developed to MII after control, CL-A, and OA exposure, respectively. These oocytes that had undergone transient PP inhibitor treatment and had developed on to MII were also capable of fertilization and early embryonic development (Fig. 3D).

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<th>Treatment</th>
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<th>24</th>
<th>48</th>
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<tr>
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<td>30</td>
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<td>0</td>
</tr>
<tr>
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<tr>
<td>TALP-Hepes + 1 µM OA</td>
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<th>Time posttreatment (h)</th>
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<td>After enhancing the percentage of oocytes undergoing GVB, transient PP inhibitor treatments did not interfere with meiotic progression to MII (Fig. 3C). There was no significant difference in the proportion of oocytes developing from GVI or MI to MII at 24 or 48 h of culture between control, CL-A, and OA treatments. The overall MII yield from GVI at 48 h of culture was 17%, 26%, and 38% in oocytes exposed to control, CL-A, or OA, respectively. By 48 h of culture, 33%, 39%, and 43% of oocytes that had undergone GVB developed to MII after control, CL-A, and OA exposure, respectively. These oocytes that had undergone transient PP inhibitor treatment and had developed on to MII were also capable of fertilization and early embryonic development (Fig. 3D).</td>
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**Oocyte PP1 and PP2A Immunohistochemistry**

Because OA and CL-A inhibit the activity of both PP1 and PP2A, the question arises whether PP1, PP2A, or both enzymes are present in the macaque oocyte. To address this question, immunohistochemistry was performed on ovarian tissue with specific antibodies against PP1 and PP2A. Mouse cardiac myocytes contain both PP1 and PP2A [10] and were utilized as a positive control (Fig. 4, B and C, respectively). PP1 and PP2A were both localized in the oocyte of preantral (Fig. 4, E and F respectively) and antral follicles (Fig. 4, H and I, respectively). Preincubation of primary antibodies with their respective antigenic peptides prior to immunohistochemical analysis abolished the signal in the oocyte (Fig. 4, h and i). Most interesting is the differential localization of PP1 and PP2A in the oocytes of both preantral and antral follicles. In these oocytes, both PP1 and PP2A were localized throughout the cytoplasm, but PP1 was highly expressed in the nucleus whereas PP2A was markedly reduced in the nuclear area.

**DISCUSSION**

The intracellular mechanisms governing regulation of mammalian oocyte meiosis have been extensively investigated, with significant progress in the identification of critical components such as cAMP [4], PKA [5], mitogen-activated protein kinase [25], and MPF [26]. However, it is becoming increasingly apparent that significant gaps in our knowledge exist with respect to regulation of these intracellular enzymes. In addition, the means by which these enzymes, and yet undefined meiosis modulators, directly influence GVB, chromosomal condensation, and assembly and association of cytoskeletal elements during meiotic progression requires elucidation. Recently, reports in both invertebrates and vertebrates have suggested that PPs also contribute to regulation of oocyte meiosis [13-19]. Evidence for a role of PPs in mammalian oocyte meiosis control is limited to findings from studies that have utilized the pharmacological, cell-permeable, complex fatty acid derivative OA [15-19, 25], which inhibits the activity of both PP1 and PP2A [27]. In all of these reports, microinjection of OA or extended culture in OA (2-16 h) stimulated GVB, yet oocytes failed to continue meiosis to MII and showed signs of cytoplasmic degradation. Results from the present study demonstrate that 1) continuous exposure of macaque oocytes to CL-A enhances oocyte GVB yet yields oocytes with significant cytoplasmic aberrations that are incapable of development to MII; 2) transient exposure of GVI oocytes to either CL-A or OA enhances GVB, does not increase cytoplasmic abnormalities, and produces oocytes capable of MII development and fertilization; and 3) macaque oocytes contain both PP1 and PP2A, which are localized differentially throughout the cytoplasm and nucleus. This is the first report directly identifying both PP1 and PP2A in mammalian oocytes.

CL-A, originally derived from the marine sponge *Discomeria calyx*, is similar to OA in its potent inhibition of PP1 and PP2A [11]. However, CL-A differs from OA in that PP1 and PP2A display greater sensitivity to CL-A, therefore requiring less pharmacological inhibitor to yield a comparable decrease in PP1/PP2A activity [11]. This has been demonstrated in cell-free assay systems, in which PP1 and PP2A are inhibited completely at approximately 1.0 nM CL-A. This difference led us to investigate the influence of CL-A on resumption of oocyte meiosis. A dose of 50 nM was selected based on previous concentrations of OA utilized (1-10 μM) [15, 17, 19] and from experience using CL-A to assess the role of PPs in primate sperm motility [12]. Macaque GVI oocytes responded to the PP1/PP2A inhibition by CL-A in a manner similar to that described in previous reports on the effects of OA on murine and bovine GVI oocytes [15-19]. Although continual treatment of macaque oocytes with CL-A significantly enhanced the percentage of oocytes undergoing GVB, the resulting oocytes did not progress in maturation due to cytoplasmic aberrations. The question arises whether this effect is due to cytotoxicity or is indicative of a biological role for PP1 and/or PP2A during maturation between GVB and MII. Similar cellular alterations have been observed in hepatocytes incubated in 1 μM OA for several hours [23]. In the latter report, hepatocytes displayed morphological changes characteristic of apoptosis, which could also describe the oocyte changes seen in the current study using CL-A and in previous studies with OA [15-19].

Because 1 μM OA completely prevents serine/threonine dephosphorylation of most proteins [24], and in view of the central role of phosphorylation/dephosphorylation in cellular regulation, it is not surprising that multiple secondary effects are observed following extended culture with this PP inhibitor. Short-term (15 min) incubation of hepatocytes with 1 μM OA did not cause morphological changes observed during extended culture nor did it adversely affect cell viability, yielding cells that retained their ability to continue activities such as glycogenolysis and gluconeogenesis [24]. We have demonstrated that macaque oocytes respond in a similar manner to short-term incubation in both CL-A and OA. Under these treatment regimens, GVI oocytes are stimulated to resume meiosis; they do not develop cytoplasmic aberrations and maintain the capability to develop to MII and fertilize. Short-term inhibition of PP1 and/or PP2A not only enhances the percentage of oocytes undergoing GVB but also enhances the rate of GVB in a subset of oocytes as indicated by the significant increase in GVB at the 1-h time point in comparison to the control value. The difference in GVB rate between CL-A and OA treatment at the 48-h time point is interesting and merits further investigation. This difference may be indicative of variations in inhibitor cell permeability, intracellular mechanisms of inhibitory activity, or localization and accessibility of intracellular PPs; all of these are poorly understood at present.

GVB rates for rhesus monkey oocytes have been reported to range from 13% to 48% [28, 29], with the majority of this meiotic resumption occurring during the first 24 h. In this study, GVB rates for the control groups in the two separate experiments were 23% and 47%. This difference in GVB rates between controls in separate experiments is not surprising and may be due to influences of season, animal age [30], and/or menstrual cycle stage [28].

The ability of oocytes to display normal morphology and continue meiotic maturation to MII after transient treatment with PP inhibitors—something that does not occur with microinjection or extended or continuous exposure—may be an important clue to the multiple roles that PP1 and/or PP2A might play in meiotic control. In the initial process of GVB, it has been suggested that PP1 and/or PP2A acts downstream of cAMP-dependent protein kinase yet prior to, or at the point of, MPF activation [16]. However, this does not preclude the possibility that PP1 and/or PP2A may also act downstream of MPF in the regulation of meiosis resumption, a possibility that requires investigation and may be supported by the recent report of cyclin-dependent
kinase regulation of skeletal PP1 activity [31]. It also appears that after reinitiation of meiosis, continual inhibition of PP1 and/or PP2A is nonsupportive of meiotic progression and causes oocytes to lose their ability to organize metaphase microtubules [16]. It has been reported previously that hyperphosphorylation of microtubule proteins, due to continual inhibition of PPs, causes depolymerization of spindle and neuronal microtubules [32]. Interestingly, one of the major intracellular sites of PP2A localization in neuronal and non-neuronal cells is associated with cytoskeletal components such as microtubules, centrosomes, and mitotic spindles [33]. This raises the question whether the oocyte 1) contains either PP1 or PP2A, with this PP having multiple regulatory check-points in meiosis; 2) possesses both enzymes, which have distinct regulatory functions; or 3) has an intricate combination of these possibilities. To begin to address this question, it is imperative to first determine whether mammalian oocytes contain PP1, PP2A, or both enzymes.

In this report we have demonstrated that macaque oocytes from preantral and antral follicles contain both PP1 and PP2A. The anti-PP1 antibody utilized in this experiment was prepared against a portion of the catalytic domain of PP1, isotype α. At present, there are five known isoforms of the catalytic subunit of PP1, termed PP1α, PP1β, PP1γ, PP1δ, and PP1ε [9]. PP1α is the major isoform characterized previously by Western blots in skeletal and heart muscle [10] and in the present study in cardiac myocytes by immunohistochemistry as a positive control. The anti-PP1 antibody also detected PP1α in the cytoplasm and nucleus of macaque oocytes. Whether this α isoform of PP1 is the only form present in the mammalian oocyte is currently under investigation. When paraffin sections included the oocyte nucleus, the intensity of PP1α immunohistochemical staining was much greater in the nucleus as compared to the cytoplasm. PP1 activity has been described in nuclear fractions obtained from hepatoma cells [34] and human MG-63 osteosarcoma cells (31). Translocation of PP1 has been described during differentiation of HL-60 cells to monocytes, at which time PP1 in the cytosolic fraction decreased as a concomitant increase occurred in the membrane and nuclear fractions [35]. Whether there is movement of PP1 in the oocyte during various stages of oogenesis and oocyte function, for example during the acquisition of meiotic competence, remains to be determined. Recently, we have found that PP1 has a similar localization pattern in fully grown mouse oocytes (unpublished results); however, oocytes from primordial follicles do not contain higher levels of PP1 in the nucleus as compared with the cytoplasm as is evident in oocytes of more developed follicles.

The presence of PP1 in the nucleus of GVB oocytes may suggest a direct regulatory role in GVB and/or chromatin condensation. Nuclear envelope breakdown, entailing disassembly of the nuclear lamina [36], requires phosphorylation of the lamins, and thus GVB, could result from an increase in phosphorylated state of nuclear lamins and nuclear membrane integrity. Increased phosphorylation of nuclear lamins, and thus GVB, could result from an increase in phosphorylated activity, a decrease in PP1 activity, or a combination of these situations. Such a mechanism may be involved in this and other investigations that have demonstrated that PP1/PP2A inhibition results in GVB. Similarly, chromatin condensation is believed to be regulated by phosphorylation of histones and other chromosomal proteins by MPF [39, 40]. Inhibition of PP1 and/or PP2A with OA also stimulates chromatin condensation during mitosis [41] and meiosis [17, 42]. This observation, combined with the stimulatory effects of OA and CL-A on oocyte meiosis resumption and the localization of PP1 in the nucleus, may indicate that PP1, along with MPF, is involved directly in the regulation of the phosphorylated state of histones and other chromosomal proteins, a possibility that merits further investigation.

Predominant localization of PP2A in the ooplasm suggests that PP2A might be associated with cytoskeletal components. The possible role of PP2A in the regulation of microtubule assembly in oocytes, similar to its role in somatic and neuronal cells [32, 33], is intriguing. Unfortunately, there are presently no pharmacological PP2A inhibitors available that do not also inhibit PP1, making this question difficult to address. Presently investigations are under way to study the ultrastructural localization of PP2A in the oocyte at various stages of nuclear maturation in an attempt to gain indirect evidence of the role of cytoplasmic PP2A in oocyte meiosis. Future experiments that might provide direct evidence of oocyte PP2A and microtubule assembly could involve suppressing PP2A activity through antisense oligonucleotide strategies or antibody neutralization with subsequent investigation of microtubule nucleation and gross oocyte cytoplasmic morphology.

In conclusion, although continual exposure of macaque oocytes to CL-A enhances GVB and results in oocytes displaying various cytoplasmic aberrations, transient treatment with CL-A or OA stimulates GVB without increasing the incidence of morphological abnormalities. These transiently treated oocytes retain the ability to develop to MII and to fertilize. In addition, we have demonstrated that macaque oocytes contain both PP1 and PP2A and that these enzymes are located predominantly in the nucleus and cytoplasm, respectively. Future studies will focus on the specific regulatory function(s) of these PPs during the process of oocyte nuclear and cytoplasmic maturation.

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REFERENCES


is a specific tyrosine phosphatase that directly activates p34\textsuperscript{cdk2}. Cell 1991; 67:197–211.


