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The Proteasome Is Involved in the First Metaphase-to-Anaphase Transition of Meiosis in Rat Oocytes[1]

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ABSTRACT

The proteasome engages in protein degradation as a regulatory process in biological transactions. Among other cellular processes, the proteasome participates in degradation of ubiquinated cyclins in mitosis. However, its role in meiosis has not been established. Resumption of meiosis in the oocyte involves the activation of maturation promoting factor (MPF), a complex of p34cdc2 and cyclin B. Inactivation of this factor, occurring between the two meiotic divisions, is associated with degradation of cyclin B. In this study, we examined the possible involvement of the proteasome in regulation of the exit from metaphase I in spontaneously maturing rat oocytes. We found that upon resumption of meiosis, proteasomes translocate to the spindle apparatus. We further demonstrated that specific inhibitors of proteasome catalytic activity, MG132 and lactacystin, blocked polar body extrusion. Chromosome and microtubule fluorescent staining verified that MG132-treated oocytes were arrested at metaphase I. Intervention of proteasomal action with this inhibitor also resulted in accumulation of cyclin B and elevated activity of MPF. These data demonstrate that proteasomal catalytic activity is absolutely essential for the decrease in MPF activity and completion of the first meiotic division. Its translocation to the spindle apparatus may facilitate the timely degradation of cyclin B.

INTRODUCTION

Meiosis in mammalian oocytes starts during embryonic life and arrests around birth at the first prophase. Meiosis resumes in the postpubertal mammalian female, when a selected number of oocytes at each sexual cycle mature into fertilizable eggs [1]. Reinitiation of meiosis is manifested by chromosome condensation, disintegration of the nuclear envelope (germinal vesicle breakdown [GVBD]), and spindle formation. The first meiotic division concludes by segregation of the homologous chromosomes and emission of the first polar body (PB). Immediately thereafter, the second meiotic division commences and is arrested again at the second metaphase (MII). Meiosis terminates upon fertilization, when sister chromatids are segregated and the second PB is extruded [2].

Oocyte maturation is triggered in vivo by a surge of the pituitary gonadotropin LH, which uncouples the oocyte from the surrounding cells of the ovarian follicle. This uncoupling results in the decrease of cAMP concentrations in the oocyte, which is a prerequisite for meiosis resumption [3]. A similar reduction in intraoocyte content of cAMP, consequent to their separation from the enveloping ovarian follicle, results in spontaneous maturation [4].

Reinitiation of meiosis represents the transition from G2 to M phase of the cell cycle and is regulated by the maturation promoting factor (MPF), a complex of the cyclin-dependent kinase, p34cdc2, and cyclin B (reviewed in [2]). Cyclin binding to p34cdc2 forms the pre-MPF complex, the activation of which is achieved by dephosphorylation of Thr-14 and Tyr-15 on p34cdc2 [5]. MPF inactivation, occurring between the two meiotic divisions, is associated with degradation of cyclin B1 [6].

Several mechanisms ensure the precise action of key regulators in the complex process of meiotic division, in which meticulous regulation prevails. Phosphorylation/dephosphorylation reactions represent a well-known mechanism for modifying protein activity. Alongside this posttranslational modification, protein degradation has been shown to participate in activation and inactivation of several signal transduction pathways [7]. One of the emerging representatives of this notion is the proteasome, which is the primary participant in the mechanism of protein degradation.

The proteasome is a multicatalytic protease that is able to hydrolyze C-terminal peptide bonds to acidic, basic, and hydrophobic amino-acid residues. It comprises approximately 1% of the protein in mammalian cells and serves as the main cellular protein degradation pathway [8, 9]. Electron microscopy of purified proteasomes from *Xenopus* oocytes reveals a large (26S) complex structure of a dumbbell shape [10], consisting of a central core catalytic subunit (20S) shaped like a cylinder, bordered by two large components (19S) at the ends of the core. Lactacystin, a *Streptomyces* metabolite, was found to inhibit proteasomal proteolysis by binding and modifying its catalytic subunit [11–13].

The idea that the proteasome participates in regulation of meiosis was initially based on reports in lower-order eukaryotes, in which changes in its catalytic activity during oocyte maturation were demonstrated [14–19]. Prevention of GVBD by inhibition of proteasomal proteolytic activity further suggested that this proteinase is involved in reinitiation of meiosis in toad and starfish oocytes [17, 19]. Other studies suggested the involvement of proteasomal action in termination of meiosis [20]. Furthermore, the establishment of the elaborate enzymatic system that leads to ubiquitin-mediated proteolysis of cyclin B [21–23] was characterized in clam oocytes entering the first meiotic division [24]. Nevertheless, the precise action of the proteasome at the transition between the two meiotic divisions has not been demonstrated. Furthermore, the involvement of the proteasome during meiosis of mammals is unknown.

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The general goal of our study was to elucidate the role of the proteasome during mammalian oocyte maturation. Our research was specifically directed at the first round of meiosis, a unique case of cell division that does not involve separation of sister chromatids but rather segregation of homologous chromosomes. We assumed that information generated by the many studies on mitosis, or even on the second round of meiosis, is not necessarily applicable to this particular example of the cell cycle. We herein report that during resumption of meiosis in rat oocytes, the proteasomes translocate to the spindle apparatus. Inhibition of the catalytic activity of the proteasome results in cyclin B accumulation, which maintains MPF activity and arrests the oocyte at metaphase I (MI) by preventing extrusion of the first PB.

MATERIALS AND METHODS

Reagents and Antibodies

Leibovitz's L-15 tissue culture medium was purchased from Gibco BRL (Paisley, Scotland). Antibiotics were purchased from Bio-Lab Ltd. (Jerusalem, Israel). MG132 (Z-leu-leu-CHO) and lactacystin were purchased from Calbiochem (La Jolla, CA). A calpain II inhibitor, LLmL (*N*-acetyl-L-leu-leu-normethioninal), leupeptin, isobutylmethylxanthine (IBMX), histone H1 (type III-S), monoclonal mouse anti-α and β-tubulin antibodies, fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse antibodies, DAPI (4′,6′-diamidino-2-phenylindole), and fetal bovine serum were purchased from Sigma (St. Louis, MO). Cy3-conjugated anti-rabbit antibodies were purchased from Jackson ImmunoResearch Laboratories Inc. (West Grove, PA). Horseradish peroxidase (HRP)-conjugated goat anti-mouse antibodies were purchased from Zymed (San Francisco, CA). Protein A-HRP, [γ-^{32}P]adenosine 5′-triphosphate (3000 Ci/mmol), and enhanced chemiluminescence (ECL) Western blotting detection reagents were purchased from Amersham (Buckinghamshire, England). Monoclonal mouse anti-cyclin B1 antibodies [25] were a kind gift of Dr. M. Brandeis (The Hebrew University, Jerusalem, Israel). The antibody was raised against residues 160–300 of hamster cyclin B1, which is 99.2% identical to rat cyclin B1 and shares no homology with other rat cyclins. Antisera against rat granulosa 20S proteasomes were raised in our laboratory [26].

Animals

Sexually immature female Wistar rats (23–25 days old) from our departmental colony received s.c. injections of 15 IU of eCG (Sanofi Sante Nutrition Animale, Libourne, France) in 0.1 ml of 0.9% NaCl for induction of follicular development. The rats were killed by cervical dislocation 48 h later. The investigation was conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, National Academy of Science, Bethesda, MD)

Oocyte Recovery and Culture

Oocytes were recovered and incubated as described previously [27]. Briefly, the oocytes were isolated into Leibovitz's L-15 tissue culture medium, supplemented with 5% fetal bovine serum, 100 IU/ml penicillin, 50 μg/ml streptomycin, and 0.25 μg/ml fungizone. Cumulus cells were removed enzymatically by collagenase (50 IU/ml, 30 min), and denuded oocytes were incubated in a 37°C humidified incubator. At the end of incubation, the oocytes were analyzed for maturation by differential interference contrast (DIC) microscopy. The presence of a germinal vesicle (GV) was used to classify oocytes as meiotically arrested. Resumption of meiosis, which was indicated by GVBD, occurred spontaneously in oocytes incubated for 4 h after their isolation from ovarian follicles. To prevent spontaneous GVBD, oocytes were incubated with the cAMP phosphodiesterase inhibitor, IBMX [28]. The first PB was emitted at 10–12 h of incubation in inhibitor-free medium, and after an overnight incubation, the oocytes were arrested at MII. For proteasomal inhibition, oocytes were released and further incubated in medium containing the proteasome inhibitors MG132 and lactacystin.

For transient exposure, the oocytes were recovered and placed into inhibitor-containing medium. After 24-h incubation, the oocytes were either placed into fresh inhibitor (continuous exposure) or washed 5 times and further incubated in inhibitor-free medium (transient exposure). The oocytes were examined morphologically after an additional 12 h.

Western Blot Analysis and Cytochemistry

At the end of the specified incubation time, the oocytes were lysed in lysis buffer (1% Triton X-100, 50 mM Hepes pH 7.2, 150 mM NaCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 1 mM PMSF, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 mM sodium-orthovanadate, 10% glycerol, 30 mM NaF, 30 mM sodium-pyrophosphate) and subjected to Western blot analysis as described previously [29]. The following antibodies were used: rabbit antisera against rat granulosa 20S proteasomes (1:1000 dilution), monoclonal mouse anti-β-tubulin antibodies (1:1000 dilution), and monoclonal mouse anti-cyclin B1 antibodies (1:750 dilution). The relevant HRP-conjugated secondary antibodies were used, and immunoreactive bands were detected by ECL. Densitometric analysis was performed using the 420oe densitometer (Pdi, Huntington Station, NY) supported by Quantity One software (Pdi).

For immunofluorescence, oocytes were fixed and immunostained with rabbit antisera against rat granulosa 20S proteasomes (1:200 dilution), incubated with the secondary Cy3-conjugated anti-rabbit antibodies (1:250 dilution) and monoclonal mouse anti-β-tubulin antibodies (1:200 dilution), and then incubated with the secondary FITC-conjugated rabbit anti-mouse antibodies (1:200 dilution) as described previously [29]. DAPI was added along with the relevant secondary antibody (1:200 dilution), or by itself. Oocytes in 50% glycerol/PBS were mounted on silicon-coated glass slides and covered by coverslips resting on a silicone ring containing 100 μm glass beads that served as spacers. The oocytes were visualized by both phase-contrast and fluorescent microscopy, using an Optiphot-2 microscope (Nikon Co., Tokyo, Japan) equipped with BP546/455 filters. Alternatively, a laser scanning confocal microscope (Zeiss, Oberkochen, Germany; LM410) was used.

H1 Kinase Activity

Histone H1 kinase activity was measured in lysates of 25 oocytes, prepared by freezing and thawing in 10 μl kinase buffer (15 mM 3-(*N*-morpholino)propanesulfonic acid [MOPS], 80 mM β-glycerophosphate, 10 mM EGTA, 15 mM $MgCl_2$, 0.1 mM PMSF, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 10 μg/ml PKI, a cAMP-dependent protein kinase inhibitor peptide). Kinase reactions were initiated by the

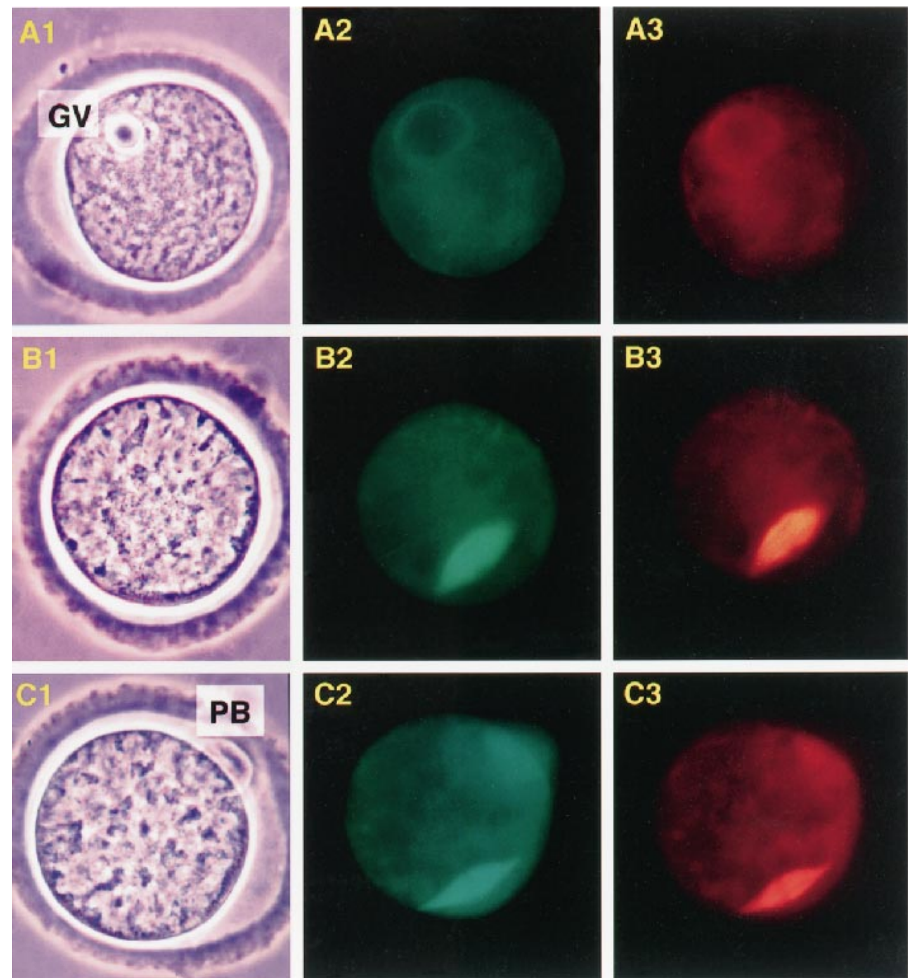

FIG. 1. Localization of proteasomes in rat oocytes during meiosis. Spontaneously maturing rat oocytes were fixed at various stages of meiosis and were immunostained with anti-20S proteasome antibodies (red) and β-tubulin (fluorescent yellow-green). **A**) A meiotically arrested GV oocyte, incubated in the presence of 0.2 mM IBMX, that has not yet assembled the spindle apparatus (**A2**), showing low concentration of proteasomes (**A3**) in the ooplasm and a relatively high perinuclear concentration around the GV. **B**) An oocyte, 8 h after isolation from ovarian follicle, resuming meiosis in MI, displaying translocation of the proteasome (**B3**) to the spindle apparatus (**B2**). **C**) A mature oocyte, after an overnight incubation, arrested at MII, displaying translocation of the proteasomes (**C3**) to the spindle apparatus (**C2**); note a low labeling associated with the first PB.

addition of 10 μl of substrate buffer (2 mg/ml histone H1, 2 mM dithiothreitol (DTT), 5 μCi [γ-^{32}P]ATP), and the reactions were carried out at 30°C for 30 min. Kinase reaction products were subjected to SDS-PAGE and autoradiography. Densitometric analysis was performed utilizing the Fujix BAS1000 phosphoimager, supported by MacBas software (Fujix, Tokyo, Japan).

RESULTS

Localization of the Proteasome in Rat Oocytes During Meiosis

Possible changes in proteasome localization during resumption of meiosis were examined by double-staining of spontaneously maturing rat oocytes with antibodies against 20S proteasomes as well as against β-tubulin. We found that in meiotically arrested oocytes the proteasomes are localized at the perinuclear region (Fig. 1, A3). Oocytes resuming meiosis exhibited low cytoplasmic concentration of the proteasomes and their clear translocation to the spindle apparatus of the first meiotic division (Fig. 1, B1–B3). Oocytes arrested at MII (Fig. 1C) exhibited as well an unequivocal colocalization of the proteasome with the MII spindle apparatus (Fig. 1, C2–C3). Some staining of the proteasome was also associated with the first PB (Fig. 1, C3).

The pattern of proteasome expression during meiotic division was examined by Western blot analysis in oocytes extracted at various stages of meiosis. Two major bands in the range of M_r 25–35 × 10^{-3}, which correspond to proteasomal subunits [29], were detected in the oocyte lysates (Fig. 2). A slight increase in the protein amount was observed upon progression from GV to GVBD. No further change in the proteasome concentration was evident in oocytes proceeding to MII.

Effect of Proteasome Inhibitors on Resumption of Meiosis

In order to characterize the function of the proteasome in meiosis, selective inhibitors of its catalytic activity were analyzed for their effect on spontaneously maturing oocytes. The oocytes were monitored morphologically for GVBD and PB extrusion.

As shown in Figure 3A, lactacystin, a potent and highly specific irreversible inhibitor of proteasomal proteolytic activity, and MG132, a potent reversible proteasome inhibitor, blocked PB extrusion at a concentration range of 1–10 μM. Inhibitors of other proteolytic pathways such as the calpain II inhibitor LLmL (Fig. 3A), as well as leupeptin, an inhibitor of lysosomal degradation (data not shown) did not inhibit PB extrusion even at a high concentration of 50 μM. Interestingly, none of the inhibitors employed in this set of experiments interfered with GVBD. Figure 3B depicts the results of testing the reversibility of the inhibition by lactacystin and MG132. Oocytes incubated for 24 h with these agents were extensively washed with control media. Further incubation was performed for 12 additional hours in the presence or absence of the inhibitors (continuous exposure

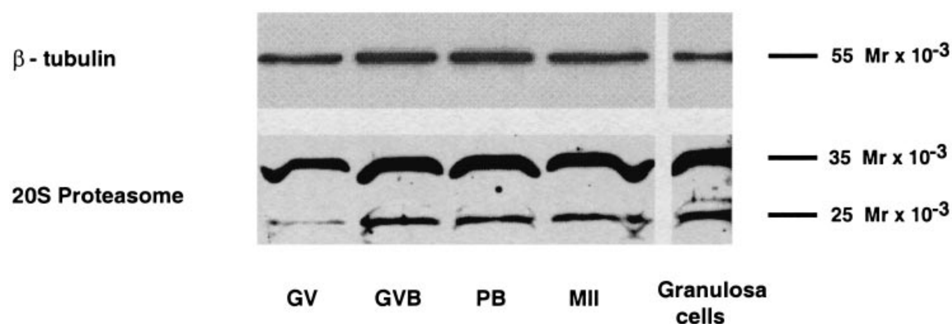

FIG. 2. The expression pattern of proteasomes during meiosis. Spontaneously maturing rat oocytes (250 per lane) were extracted at various stages of meiosis as follows: GV, incubated in the presence of 0.2 mM IBMX, GVBD (3–4 h after isolation), PB (10–12 h after isolation), and MII (after overnight incubation). The right lane contains an equivalent amount of protein extracted from rat granulosa cells. The extracts were separated and immunolabeled with anti-20S proteasome antibodies. Anti-β-tubulin antibodies were used in order to normalize for protein concentration. The experiment was repeated 4 times; the results of one representative experiment are presented.

and transient exposure, respectively). The inhibition of PB extrusion by MG132 was reversible whereas that induced by lactacystin was not (Fig. 3B).

The morphology of the arrested oocytes presented a particular interest. Oocytes incubated in inhibitor-free medium extruded the PB as expected, 10 h after their isolation (Fig. 4A). In contrast, the MG132-treated oocytes that failed to extrude the PB exhibited an elongated protrusion (Fig. 4B), which was evident throughout the incubation of the oocytes with the drug.

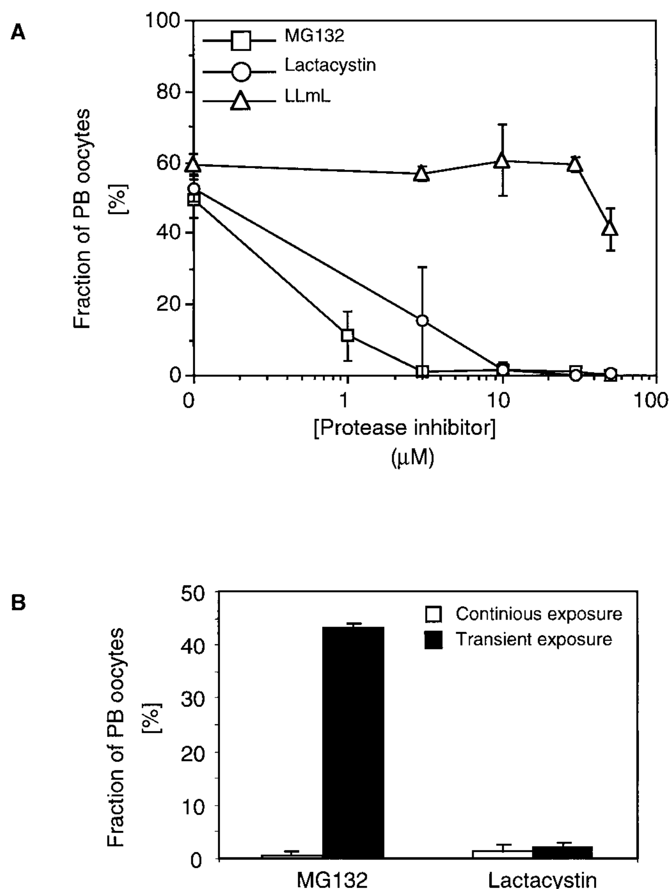

FIG. 3. **A**) The effect of proteasomal activity inhibition on PB extrusion. Rat oocytes were incubated for 24 h with MG132 (squares), lactacystin (circles), and LLmL (triangles). The fraction of oocytes that extruded the first PB is presented. The means of at least 3 different experiments (minimum of 160 oocytes for each experimental point) are presented along with their standard errors. **B**) Recovery from the effect of the proteasome inhibitors. Oocytes incubated for 24 h in 10 μM of either MG132 or lactacystin were extensively washed and further incubated for 12 h in the presence (continuous exposure) or absence (transient exposure) of the inhibitor. The fraction of oocytes that extruded a PB is presented. Means of at least 3 different experiments (minimum of 160 oocytes for each experimental point) are presented along with their standard errors.

In order to identify the time during meiosis at which the proteasome-sensitive event takes place, oocytes were isolated in inhibitor-free medium, and MG132 was added at different times during incubation. We found that addition of MG132 at any time, even just before PB extrusion, effectively prevented its emission (data not shown). The precise stage of inhibition throughout meiosis was confirmed by chromosome staining. Control, MII-arrested oocytes incubated in inhibitor-free medium for 24 h showed two stained DNA aggregates, indicating segregation of chromosomes between the oocyte and PB (Fig. 5A′). On the other hand, the MG132-treated oocytes exhibited only one stained aggregate localized in the elongated protrusion, suggesting failure of chromosome segregation and incomplete metaphase-to-anaphase transition of MI (Fig. 5B′).

Double staining of chromosomes and microtubules corroborated that oocytes were experimentally arrested at the first metaphase-to-anaphase transition. Whereas control oocytes presented chromatids arranged on the second metaphase spindle (Fig. 6A) with the remnants of their homologues in the PB (Fig. 6A′), MG132-treated oocytes showed incomplete segregation of the chromosomes on the first meiotic spindle (Fig. 6B). Chromosomes that were partially segregated but still localized on the metaphase plate were also observed in many oocytes examined by conventional microscopy. Nevertheless, anaphase was never observed.

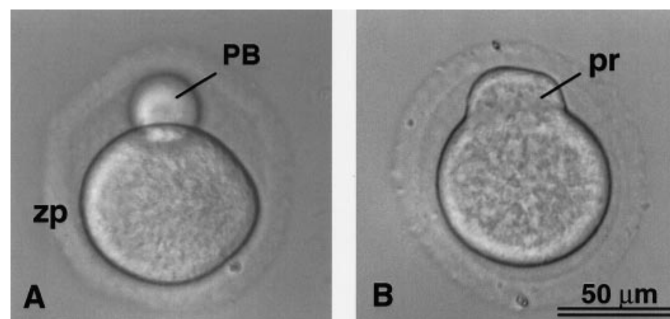

FIG. 4. Morphology of MG132-arrested oocytes. Rat oocytes were incubated in the absence (**A**) or presence (**B**) of MG132 and were monitored with DIC microscopy. **A**) First PB extrusion in control oocytes incubated for 24 h. zp, Zona pellucida. **B**) Inhibition of PB extrusion by MG132; note the elongated protrusion (pr).

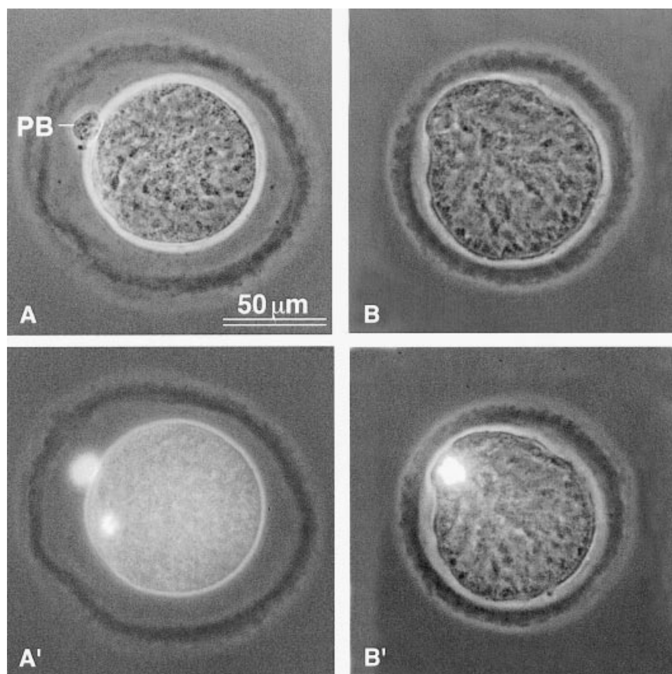

FIG. 5. Cellular localization of DNA in MG132-arrested oocytes. Rat oocytes incubated in the absence (**A, A′**) or presence (**B, B′**) of MG132 were fixed and stained for DNA (DAPI staining) and examined with phase contrast (**upper**) or fluorescent microscopy (**lower**). **A**) Extrusion of the first PB in an oocyte incubated in inhibitor-free medium for 24 h. **A′**) Note segregation of chromosomes between the oocyte and PB. **B**) MI-arrested oocyte after 24 h incubation with MG132 (30 μM). **B′**) Note failure of chromosomes to segregate.

Degradation of Cyclin B by Proteasomes

The fact that exit from first meiosis was prevented by the proteasome inhibitors suggested that cyclin B1 could be a likely candidate for proteasome degradation. To test this hypothesis, we subjected extracts of control and MG132-treated oocytes to Western blot analysis using anti-cyclin B1 antibodies. Figure 7 depicts a relatively small amount of cyclin B1 in control oocytes that extruded the first PB after 10 h of incubation, reflecting the degradation of cyclin B1 between the two meiotic divisions. MG132-treated oocytes at 10-h incubation time expressed higher amounts of this protein (Fig. 7, upper panel). Densitometric analysis disclosed a 2.9-fold increase in the amount of this protein in MG132-treated oocytes compared to controls after 10 h of incubation (Fig. 7, lower panel). Interestingly, during the second meiotic division, in which cyclin B1 is synthesized preceding the second metaphase, the same pattern of cyclin accumulation occurred. Oocytes that were incubated with MG132 for 24 h accumulated more cyclin B1 than control, MII-arrested oocytes. In a further experiment, we found that the amount of cyclin B1 was lower in oocytes recovering from MG132 inhibition than in oocytes continuously exposed to this inhibitor (data not shown). No changes were observed in the amount of tubulin in the oocytes incubated under the same conditions.

The accumulation of cyclin B was corroborated by measurements of MPF activity in the presence or absence of MG132. MPF was monitored in oocytes resuming meiosis by a histone H1 kinase assay—an assay that is routinely used for monitoring p34cdc2 kinase activity. Analysis of H1 kinase activity, under conditions of proteolytic inhibition, revealed that the addition of MG132 resulted in a relatively higher kinase activity at the time of first PB extrusion. In control oocytes that had just extruded a PB (10 h after isolation), the amount of phosphorylated histone H1 was relatively low, representing the inactivation of MPF between the two meiotic divisions, due to cyclin B degradation (Fig. 8). Inhibition of proteolytic activity in oocytes by incubation in MG132 for 10 h resulted in a significant, 2.8-fold increase in MPF activity (Fig. 8, lower panel). An increase in the amount of phosphorylated histone H1 under conditions of proteolytic inhibition was also evident at the second meiotic division. The addition of MG132 resulted in a relatively higher kinase activity in the treated oocytes as compared to control, MII-arrested oocytes at 24-h incubation time.

DISCUSSION

In this study, we examined the role of protein degradation by the proteasome in rat oocytes resuming meiosis. Our study provides the first demonstration that inhibition of the catalytic activity of the proteasome arrests the oocyte at MI, preventing PB extrusion. It further shows that these MI-arrested oocytes accumulate cyclin B and maintain a high level of MPF activity. It also reveals that throughout resumption of meiosis, proteasomes translocate to the spindle apparatus. Our study strongly suggests that proteasomal

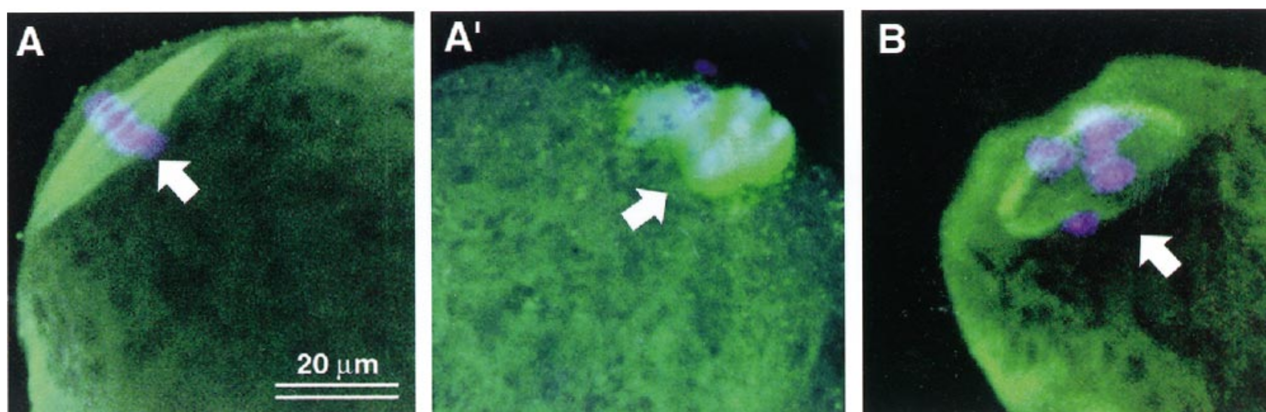

FIG. 6. Cellular localization of DNA and microtubules in MG132-arrested oocytes. Images of spindles from oocytes incubated in the absence (**A, A′**) or presence (**B**) of MG132 (10 μM) for 24 h, double-stained for β-tubulin (fluorescent green) and DNA (blue), and examined with confocal microscopy. **A**) The chromatids are arranged at the MII plate, in a longitudinal section of the spindle. **A′**) High concentration of tubulin in the PB with the remnants of the homologous chromosomes. **B**) Chromosomes arrested in metaphase/anaphase I; a diagonal section of the spindle.

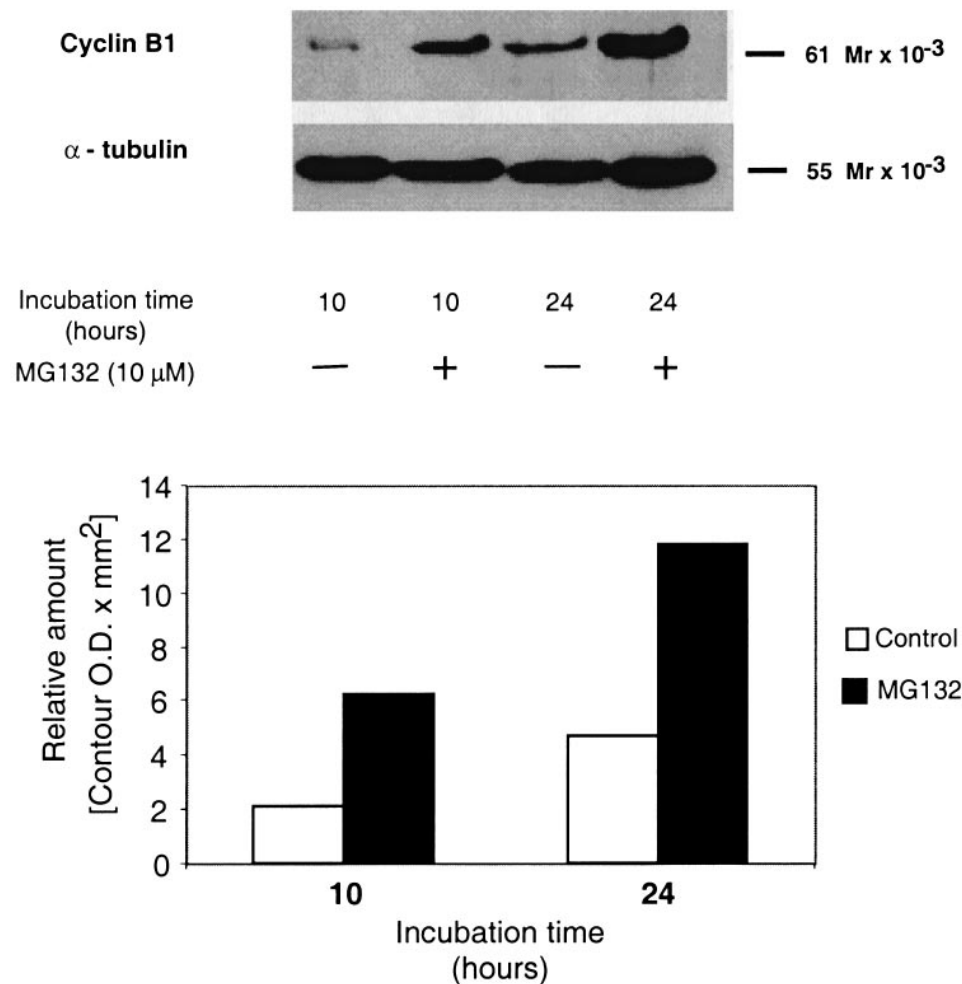

FIG. 7. The effect of MG132 on cyclin B1 level of expression. Spontaneously maturing rat oocytes (250 per lane) were extracted at the indicated times of incubation in the presence or absence of MG132 (10 μM). The extracts were separated and immunolabeled using anti-cyclin B1 and α-tubulin antibodies. The experiment was repeated 5 times. **Upper**) Results of one representative experiment; **lower**) the densitometric analysis of this experiment.

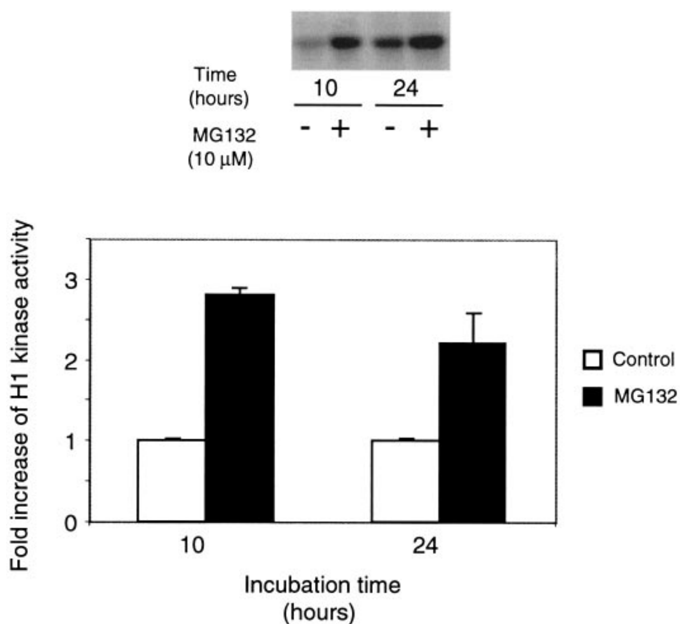

FIG. 8. The effect of MG132 on H1 kinase activity during maturation of rat oocytes. Spontaneously maturing rat oocytes (25 per lane) were extracted at the indicated times of incubation in the presence or absence of MG132 (10 μM) and assayed for H1 kinase activity. **Upper**) Results of one representative experiment; **lower**) means and SE of pooled results from 3 individual experiments.

activity is critical for completion of meiosis in rat oocytes. Furthermore, its localization around the spindle apparatus could facilitate degradation of the relevant cellular substrate.

The spindle assembly checkpoint is a part of the surveillance mechanism that monitors the completion of critical cell cycle events and allows the subsequent cell cycle transition to occur [30–32]. The translocation of proteasomes to the spindle apparatus strongly suggests that proteolysis serves as an effector of spindle function in meiotic division. This possibility is further strengthened by our finding that inhibitors of proteasomal catalytic activity arrest the oocytes at MI, preventing the completion of the first round of meiosis. In addition, the ability of proteasome inhibitors to block PB extrusion at any time point suggests that the proteasome-sensitive event occurs shortly before the metaphase/anaphase transition. This conclusion is supported further by our observation that MG132-treated oocytes exhibited an elongated protrusion, reflecting their unsuccessful ''effort'' to emit a PB. Therefore, the MI arrest implies that, at least in the rat, proteasomes degrade proteins that are crucial for anaphase to occur. Taken together, these results point towards completion of the first round of meiosis as a specific cellular event dependent on proteolysis.

Interestingly, in the rat, in contrast to lower eukaryotes [17, 19], none of the proteolytic inhibitors affected early meiotic events, such as GVBD. This inconsistency can be added to other differences between meiosis of rat oocytes

and those of lower organisms. One such difference is manifested at the very early stage of meiosis reinitiation. Rat oocytes resume meiosis spontaneously upon their separation from the follicle [33], whereas in lower organisms an external stimulatory ligand (progesterone, 1-methyladenine, or sperm) is absolutely necessary for their exit from G2-arrest.

Earlier studies have demonstrated the proteasomal destruction of mitotic cyclins [34–36], which is mediated via the ubiquitin pathway [21–23]. Direct evidence of proteasomal digestion of cyclin B was only recently presented in a cell-free system. Tokumoto et al. [20] reported that purified 26S proteasomes were shown to cleave recombinant cyclin B1 of goldfish and *Xenopus* oocyte extracts. However, that study failed to demonstrate that cyclin B cleavage affected MPF kinase activity. Our study shows for the first time a proteasome-dependent degradation of cyclin B in intact oocytes that clearly correlates with a decreased activity of MPF.

Similar to our findings in meiosis, the timely degradation of cyclin B1 was shown to be necessary for exit from mitotic M phase [21]. However, nondegradable mutants of cyclin B1 did allow separation of sister chromatids, arresting the cell cycle in telophase rather than anaphase [37–39]. These studies suggest that proteolysis of proteins other than cyclin B1 could be the direct cause of metaphase-to-anaphase transition [32]. The other candidate proteins for degradation by the ubiquitin-dependent pathway are INCENP [40] and CLiP [41], which are positioned between sister chromatids before anaphase [42]. Mammalian homologues of yeast PDS1 and CUT2, both of which are degraded during anaphase and arrest sister chromatid segregation in their nondegradable form, could also serve as candidate proteins for proteasomal action at the completion of cell division [43]. Indeed, very recently such a protein, vSecurin, was identified in vertebrates and found to be degraded by the proteasome [44]. A nondegradable mutant form of this protein blocked sister chromatid separation in *Xenopus* cycling egg extracts [44]. However, as mentioned previously, unlike the case in mitosis and the second round of meiosis, completion of the first meiotic division involves separation of homologous chromosomes. In this unique case of cell division, sister chromatids should be held together. Therefore, to avoid aneuploidy, the above-mentioned proteins should be protected from degradation and cannot serve as candidates for proteasomal degradation activity. Whether other as yet unidentified proteins are destroyed at the metaphase-to-anaphase transition of the first meiotic division of the oocyte, allowing segregation of the homologous chromosomes, should be examined.

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