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Meiotic Maturation of the Mouse Oocyte Requires an Equilibrium between Cyclin B Synthesis and Degradation

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Among the proteins whose synthesis and/or degradation is necessary for a proper progression through meiotic maturation, cyclin B appears to be one of the most important. Here, we attempted to modulate the level of cyclin B1 and B2 synthesis during meiotic maturation of the mouse oocyte. We used cyclin B1 or B2 mRNAs with poly(A) tails of different sizes and cyclin B1 or B2 antisense RNAs. Oocytes microinjected with cyclin B1 mRNA showed two phenotypes: most were blocked in MI, while the others extruded the first polar body in advance when compared to controls. Moreover, these effects were correlated with the length of the poly(A) tail. Thus it seems that the rate of cyclin B1 translation controls the timing of the first meiotic M phase and the transition to anaphase I. Moreover, overexpression of cyclin B1 or B2 was able to bypass the dbcAMP-induced germinal vesicle block, but only the cyclin B1 mRNA-microinjected oocytes did not extrude their first polar body. Oocytes injected with the cyclin B1 antisense progressed through the first meiotic M phase but extruded the first polar body in advance and were unable to enter metaphase II. This suggested that inhibition of cyclin B1 synthesis only took place at the end of the first meiotic M phase, most likely because the cyclin B1 mRNA was protected. The injection of cyclin B2 antisense RNA had no effect. The life observation of the synthesis and degradation of a cyclin B1–GFP chimera during meiotic maturation of the mouse oocyte demonstrated that degradation can only occur during a given period of time once it has started. Taken together, our data demonstrate that the rates of cyclin B synthesis and degradation determine the timing of the major events taking place during meiotic maturation of the mouse oocyte.

Key Words: **cyclin B; meiosis; synthesis; degradation; polyadenylation; mRNA; mouse oocyte.**

INTRODUCTION

In mammals, oocytes are arrested at the diplotene stage of the first meiotic prophase. Meiotic maturation is controlled through activation of the major cell cycle kinase, MPF (M-phase promoting factor), composed of a catalytic subunit, $p34^{cdc2}$, and a regulatory subunit, cyclin B (Gautier *et al.*, 1990; Draetta *et al.*, 1989; Labbé *et al.*, 1989; Meijer *et al.*, 1989). MPF is activated at GVBD (germinal vesicle breakdown). Then MPF activity rises until it reaches a plateau at the end of the first meiotic M phase (M I) (Choi *et al.*, 1991; Verlhac *et al.*, 1994). A transient decline in MPF activity takes place during the transition between meiosis I and meiosis II. MPF is reactivated rapidly to enter meiosis II (M II) and is maintained at a high level during the metaphase II arrest.

The modulation of cyclin concentration by synthesis and degradation is of central importance for the control of MPF activity (Murray and Kirschner, 1989). The mitotic cyclins are synthesized throughout the cell cycle and destroyed during a short period, just before the metaphase–anaphase transition (Evans *et al.*, 1983). Cyclin B is degraded by the ubiquitin pathway (Glotzer *et al.*, 1991; Hershko *et al.*, 1991). In mouse oocyte, the synthesis of cyclin B increases progressively during meiotic maturation, reaching its maximum at the end of the first meiotic M phase (Hampl and Eppig, 1995a; Winston, 1997). Cyclin B is then degraded at the time of polar body extrusion. Cycloheximide-treated oocytes undergo GVBD normally, because of a preexisting

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pool of cyclin B, but are unable to get a further increase in MPF activity and to extrude the first polar body (Hampl and Eppig, 1995b; Hashimoto and Kishimoto, 1988). When protein synthesis is inhibited at the end of M I, the polar body is normally extruded but MPF is not reactivated and the oocyte enters interphase (Clarke and Masui, 1983; Hashimoto and Kishimoto, 1988).

The role of cyclin B1 synthesis in the control of the duration of meiotic maturation was shown in two strains of mice, CBA/Kw and KE, which differ greatly in the timing of meiotic maturation. CBA/Kw oocytes extrude the first polar body about 7 h after GVBD while KE oocytes take approximately 3–4 h longer. The rate of cyclin B1 synthesis during M I is higher in CBA/Kw than in KE oocytes and increasing cyclin B1 synthesis in KE oocytes speeds up first polar body extrusion. Finally, the overall level of protein synthesis and the amount of cyclin B1 messenger RNA are identical in both strains, suggesting that cyclin B1 translation is controlled differently in these two strains (Polanski *et al.*, 1998). Among the different mechanisms that control the expression of maternal mRNAs, polyadenylation has been implicated in cyclin B1 translation in *Xenopus* and mouse oocytes (Barkoff *et al.*, 2000; de Moor and Richter, 1999; Tay *et al.*, 2000).

Thus, it seems that the amount of cyclin B is tightly regulated during meiotic maturation to control the timing of the various event taking place during this period. We designed experiments in which we attempted to modulate the level of cyclin B synthesis by microinjecting different sense and antisense cyclin B RNAs in immature mouse oocytes in order to interfere with meiotic maturation. The oocytes were then followed using a videomicroscope to determine precisely the timing of meiosis resumption, polar body extrusion (PBE), and entry into meiosis II. Finally, using a human cyclin B1–GFP construct, cyclin B1 synthesis and degradation were directly observed in living mouse oocytes.

MATERIALS AND METHODS

Collection and Culture of Oocytes

In this paper we used two strains of mice: CBA/Kw oocytes and KE oocytes. We used KE oocytes to overexpress cyclin B and CBA/Kw oocytes to inhibit cyclin B. As the level of cyclin B1 was low in KE oocytes, it was easier to modulate the level of this one. On the contrary, inhibition of the cyclin B1 level in CBA/Kw oocytes, where H1 kinase activity is high, can give more significant results than a strain where the level of cyclin B1 is already low.

To obtain immature oocytes arrested at prophase I of meiosis, the ovaries were removed from 5- to 6-week-old KE or CBA/Kw female mice (bred in the laboratory) or Swiss female mice (Animalerie Spécialisée de Villejuif, Centre National de la Recherche Scientifique, France). The ovaries were transferred to prewarmed (37°C) M2 medium, supplemented with 4 mg/ml bovine serum albumin (BSA) (Whittingham, 1971) and 50 μg/ml dibutyryl cyclic AMP (dbcAMP), which prevents immature oocytes from undergoing GVBD. The ovarian follicles were punctured to release the enclosed oocytes, and immature oocytes displaying a germinal vesicle (GV) were collected (oocytes from all strains used in this study have similar sizes). Samples for histone H1 kinase activity were collected in M2 medium + 4 mg/ml polyvinylpyrrolidone (PVP) and frozen immediately at −70°C.

Metaphase II-arrested oocytes were recovered from mice superovulated by intraperitoneal injections of pregnant mare's gonadotrophin (PMSG; Intervet) and human chorionic gonadotrophin (hCG; Intervet) 48 h apart. Ovulated oocytes were released from the ampullae of oviducts 13.5 h post-hCG. The cumulus cells were dispersed by brief exposure to 0.1 M hyaluronidase (Sigma).

Immunofluorescence

The fixation and labeling of oocytes were performed as described in Kubiak *et al.* (1993). We used the rat monoclonal antibody YL1/2 specific for tyrosinated α-tubulin (Kilmartin *et al.*, 1982). As second layer, we used a fluorescein-conjugated anti-rat antibody (Miles). The chromatin was visualized using propidium iodide (Molecular Probes; 5 mg/ml in PBS). Samples were observed with a Leica TCS4D confocal microscope.

Immunoblotting

Samples were collected in sample buffer (Laemmli, 1970) and heated to 100°C for 3 min. The proteins were separated by electrophoresis in 10% polyacrylamide (ratio acrylamide/bisacrylamide, 100/1) containing 0.1% SDS and electrically transferred to nitrocellulose membranes (Schleicher & Schuell; pore size, 0.45 μm). Following transfer and blocking for 2 h in 3% skimmed milk in 10 mM Tris (pH 7.5)/140 mM NaCl (TBS) containing 0.1% Tween 20, the membrane was incubated overnight at 4°C with the anti-cyclin B1 antibodies (Serotec, 1:500) diluted in blocking solution. After three washes of 10 min each in 0.1% Tween 20/TBS, the membrane was incubated for 1 h at room temperature with an anti-rabbit antibody conjugated to horseradish peroxidase (Amersham) diluted 1:1000 in 3% skimmed milk in 0.1% Tween 20/TBS. The membrane was washed three times in TBS/Tween and then processed using the Super Signal (Pierce) detection system.

Kinase Activity

Histone H1 kinase activity was determined as described by Félix *et al.* (1989) in HK buffer (80 mM β-glycerophosphate, 20 mM EGTA, pH 7.3, 15 mM MgCl2, 1 mM DTT, 1 mM PMSF, 10 μg/ml leupeptin, 10 μg/ml pepstatin, 10 μg/ml aprotinin) using exogenous histone H1 (HIII-S from calf thymus, Sigma) as the substrate. Samples containing the oocytes in medium M2 + PVP were lysed by freezing and thawing three times, diluted twice in two times concentrated HK buffer, and incubated for 15 min at 37°C in the presence of 3.3 mg/ml histone H1, 1 mM ATP, and 0.25 mCi/ml [^{32}P]ATP (Kubiak *et al.*, 1991).

The kinase reaction was stopped by adding 2× sample buffer (Laemmli, 1970) and boiling for 3 min. The samples were then analyzed by electrophoresis in 15% polyacrylamide gels containing 0.1% SDS followed by autoradiography.

The autoradiographs were digitized using an Agfa Arcus Plus scanner and the kinase activity present in each sample was quantified using NIH Image (v. 1.60) analysis software.

Plasmids

Plasmids for sense mRNAs. The full-length cDNA encoding the mouse cyclin B1 (Chapman and Wolgemuth, 1992) was subcloned from pBluescript SK(−) into pSP64(A) vector (30 A residues) (Promega) as follows. The cDNA was amplified by PCR (advantage 2 PCR, Clontech) with a 5′ primer (5′-tagcttctctagacacgaggtggatagccagag-3′) and a 3′ primer (5′-tagcggatccaaaatgagaagtcacaacct-3′). The resulting PCR was digested with *Xba*I and *Bam*HI and ligated at the *Xba*I and *Bam*HI sites of pSP64(A).

The plasmid containing the human cyclin B1–Mm GFP cDNA has been previously described (Hagting *et al.*, 1998). It was linearized by *Sfi*I and the 3′-overhang was further filled with T4 DNA polymerase. This plasmid allows *in vitro* transcription of the human cyclin B1–GFP using T3 RNA polymerase.

Plasmids for antisense RNAs. To obtain the clone corresponding to the 5′ portion of the cyclin B1 mRNA (pBluescript cyclin B1 5′), a 406-pb *Eco*RI–*Hin*dIII fragment (position 1 to 406) was cloned between the *Eco*RI and *Hin*dIII sites of pBluescript SK(−) vector (Stratagene).

The open reading frame (ORF) portion of cyclin B1, a 582-bp *Eco*RI–*Eco*RV fragment (position 1–583), was cloned between *Eco*RI and *Eco*RV. By linearization at the *Sma*I position, we could *in vitro* transcribe a 400-pb fragment (pBluescript cyclin B1 ORF).

pRN3Mos was constructed by RT-PCR amplification from mouse ovaries. Total RNA was extracted using RNeasy mini-kit (Qiagen) and 500 ng of RNA was treated with 2 U of RQ1 DNase (Promega) for 20 min at 37°C and then heated for 5 min at 85°C. First-strand cDNA synthesis was then performed with 50 U of MMuLV Superscript (Life Technologies) using 2.5 μM random hexamer (pdN6, Pharmacia)/1 mM dNTPs (Promega), in 10 mM Tris–HCl, pH 8.3, 5 mM $MgCl_2$, and 50 mM KCl for 1 h at 37°C, followed by 5 min at 95°C. The PCR amplification was then performed on 50 ng of cDNA using 5′-gatcagatctcaccatgccttcgcctctaagcc and 5′-gatcgaattctcagcctagtgcccctcggaaag primers. The 1-kb PCR product was then digested by *Bgl*II/*Eco*RI and cloned into pRN3 (Brunet *et al.*, 1998).

RNA Synthesis

To obtain cyclin B1 and B2 mRNAs with a long poly(A) tail (A(n)), pBluescript vectors containing the full-length cyclin B1 or B2 cDNAs were linearized with *Xho*I and *in vitro* transcribed with T3 RNA polymerase to produce capped mRNA (mMessage mMachine, Ambion). The cyclin B1 and B2 mRNAs were polyadenylated *in vitro* by incubation in a polyadenylation mixture: 250 mM NaCl, 50 mM Tris, 10 mM $MgCl_2$, 100 mg/ml BSA, 2 mM DTT, 1 U/ml RNAsin (Promega), 100 mM ATP, 2.5 mM $MnCl_2$, and 0.05 U/ml PolyApolymerase (Pharmacia Biotech) for 37°C as described in Vassalli *et al.* (1989). The reaction was stopped by 25 mM EDTA.

To produce cyclin B1 mRNA with a short poly(A) tail (A(30)) or with no poly(A) tail (A(0)), we linearized pSP64(A) vector containing the full-length cyclin B1 with *Eco*RI and *Bam*HI, respectively. Capped mRNAs were synthesized with SP6 RNA polymerase (mMessage mMachine, Ambion).

The full-length cyclin B1 or B2 antisense RNAs were linearized at the *Eco*RI position of pBluescript vectors containing cyclin B1 or B2 cDNAs. The pBluescript cyclin B1 5′ portion and the pBluescript cyclin B1 ORF were linearized with *Eco*RI and *Sma*I, respectively. The two antisense mRNAs against the 3′ portion were obtained by linearization at the *Sph*I (194 pb) and the *Hin*cII (468 pb) positions. All antisense RNAs were synthesized with T7 RNA polymerase (Ambion).

All samples were extracted with the RNeasy Kit (Qiagen) and resuspended in injection buffer (10 mM Tris, pH 7.4). The concentration of RNA was given in arbitrary units.

Microinjection

The *in vitro* synthesized sense and antisense RNAs were microinjected into the cytoplasm of GV oocytes using an Eppendorf pressure microinjector and sterile pipets. About 10 pl of the RNA solution containing around 0.5–1 μg/μl was injected per oocyte. We proposed to define the concentration in arbitrary units (A.U.). Five arbitrary units was the maximal concentration. The oocytes were kept in M2 medium supplemented with dbcAMP during the injection period. In certain experiments microinjected oocytes were maintained during 15 h in dbcAMP. The resumption of meiotic maturation was triggered by removal of the injected oocytes from the dbcAMP-containing medium. They were then cultured in M2 medium under paraffin oil at 37°C, in an atmosphere of 5% CO_2 in air.

RT-PCR Assay

Oocytes were collected in H_2O treated with 0.1% diethyl pyrocarbonate (H_2O–DEPC) and stored at −80°C. Samples corresponding to 50 oocytes in 10 μl were lysed by freezing and thawing. The genomic DNA from 5 oocytes was digested by adding 2 U of RQ1 DNase (Promega) in 20 μl of the reverse transcriptase mix (10 mM Tris–HCl, pH 8.3, 5 mM $MgCl_2$, 50 mM KCl, 1 mM each dNTPs (Promega), 2.5 μM random hexamer ($pd(N)_6$, Pharmacia) and 20 U of RNase inhibitor (RNAsin, Promega) and incubating at 37°C for 10 min. The reaction was stopped by heating to 85°C for 5 min. Then, the samples were supplemented with 50 U of SuperScript RNAse H–reverse transcriptase (Gibco BRL) to synthesize the cDNA. After 1 h of incubation at 37°C, the reaction was stopped by heating to 95°C for 5 min. PCR amplification was performed in the same tube in a final volume of 50 μl containing 10 mM Tris–HCl, pH 8.3, 2.5 mM $MgCl_2$, 50 mM KCl, 1 μM 5′-specific primer, 1 μM 3′-specific primer (5′-actccctgcttcctgttat-3′ and 5′-aaaatgagaagtcacaacc-3′ for cyclin B1; 5′-gctagattttggaggagccatggc-3′ and 5′-ctagggatccacggaattagacaa-3′ for cyclin B2), and 2.5 U of Taq polymerase (Perkin–Elmer). Samples were denatured at 94°C for 3 min, then subjected to 40 cycles of amplification (94°C, 1 min; 55°C, 1 min; 72°C, 1 min), and finally incubated for 10 min at 72°C. Thirteen microliters of the amplified mixture was run on 2% agarose gels. The gels were stained with ethidium bromide (5 μg/ml) and photographed under UV illumination.

Time-Lapse Fluorescence Imaging and Analysis

Microinjected oocytes were cultured under paraffin oil in a specially designed chamber at 38°C. Oocytes were observed using a motorized inverted microscope (Leica) coupled to a high-sensitivity CCD camera (Micromax Princeton) and connected to a computer that controls the different preestablished parameters (Metamorph). Acquisitions were performed using phase contrast to follow oocyte maturation (illumination, 50 ms). The acquisitions were performed every 10 min.

For human cyclin B1–GFP mRNA-microinjected oocytes, acquisitions were performed alternatively using phase contrast (illumination, 50 ms) and epifluorescence to follow cyclin B1 fluorescence (3-s illumination using a log 1 neutral density filter). The acquisitions were performed every 20 min. Images were analyzed using

TABLE 1

Effect of Cyclin B1 mRNA Injection on Meiosis Resumption and First Polar Body Extrusion in KE Oocytes in Medium Containing dbcAMP

Microinjected mRNA	% GVBD[a] (mean ± SD)	% PBE[a] (mean ± SD)	*n* (Expt)[b]
Control (none)	0	0	232 (7)
Cyclin B1-A(0)	0	0	116 (4)
Cyclin B1-A(30)	0	0	24 (1)
Cyclin B1-A(n)	36 ± 6.3	16.7 ± 3.8	277 (6)

[a] Using the nonparametric Mann–Whitney test.

[b] Number of oocytes. Number of experiments in each group is given in parentheses.

NIH Image. The area to be measured was drawn by hand on the first image of a series and reproduced on each successive image. The sum of pixel intensities was calculated in the area of interest for each image.

RESULTS

Cyclin B1 Induces Meiosis Resumption in the Presence of dbcAMP

To assay the role of cyclin B1 in the first event of meiotic maturation we overexpressed cyclin B1 by microinjecting cyclin B1 mRNA with a long poly(A) tail of about 150–300 adenosine residues after polyadenylation *in vitro* (Wormington, 1991) in KE oocytes. The microinjected oocytes resumed meiosis as the controls (73 ± 15.7% versus 73 ± 15.5%), but when we left the oocytes for a long time in dbcAMP, the cyclin B1-A(n) microinjected mRNA was able to induce GVBD in the presence of this inhibitor medium (Table 1). After 5 h of culture in the presence of dbcAMP, GVBD took place in 28% (in 36.82% 10 h later) of the oocytes injected with cyclin B1-A(n). This suggests that cyclin B1 synthesis may regulate GVBD, although it is dispensable (Stern *et al.*, 1972; Wassarman *et al.*, 1979).

Then, we microinjected cyclin B1 mRNA with shorter poly (A) tails: cyclin B1-A(30) had a short poly(A) tail (30 adenosines residues) and cyclin B1-A(0) had no poly(A) tail. The oocytes microinjected with these two forms of mRNA resumed meiosis as the controls but, in contrast to the cyclin B1-A(n) mRNA-injected oocytes, they remained arrested at the GV stage in the presence of dbcAMP. Thus, polyadenylation seems to control the level of cyclin B1 synthesis since only the oocytes microinjected with the cyclin B1-A(n) were able to synthesize enough cyclin B1 to allow the formation of a small amount of active MPF able to trigger GVBD. This was confirmed by immunoblot analysis when we observed that the amount of cyclin B1 present in the oocyte increased with the length of the poly(A) tail of the injected mRNA (Fig. 1).

Cyclin B1 Controls the Timing of the First M Phase and Polar Body Extrusion through the Level of Cyclin B1 Translation

The length of the poly(A) tail seemed to control the level of cyclin B1 synthesis. First, we followed first polar body extrusion in KE oocytes microinjected with the different cyclin B1 mRNAs that were immediately removed from the dbcAMP-containing medium. Two phenotypes were observed depending on the size of the poly(A) tail (Table 2). Many oocytes were blocked in M I, depending on the length of the poly(A) tail: the longer the poly(A) tail the more M I is blocked. The others bypassed this block and extruded their polar bodies earlier than did the controls. This advance was correlated with the length of the poly(A) tail (Table 2): the longer the poly(A) tail, the earlier the PBE. Microinjection of an unrelated mRNA (ezrin) did not influence significantly the timing of polar body extrusion (Table 2).

Most oocytes that broke down their GV in the presence of dbcAMP were unable to extrude their first polar body (Table 1) and were arrested at the metaphase stage (Fig. 2). The oocytes injected with the cyclin B1-A(n) mRNA that remained blocked at the GV stage after 15 h in dbcAMP underwent GVBD only after dbcAMP removal and extruded their polar bodies (PB) in a lower proportion when compared to controls (31% versus 93%). These experiments suggest that the higher the cyclin B1 level was, the more important the M I block. This block was likely due to the saturation of the cyclin degradation machinery (Holloway *et al.*, 1993; Van der Velden and Lohka, 1993; Luca *et al.*, 1991).

To test this hypothesis, we attempted to increase the activity of the cyclin B1 degradation machinery using ethanol treatment, a procedure used to parthenogenetically activate mouse oocytes. Oocytes injected with cyclin B1-A(n) mRNA that were blocked in dbcAMP overnight were removed from dbcAMP and separated into two groups. In the control group that was not treated with ethanol, 29.6 ± 9.5% of the oocytes extruded their PB. The control oocytes that did not extrude their PB were blocked in M I (Fig. 3A) with paired homolog chromosomes (Fig. 3B). Of the oocytes treated with ethanol at the normal time of PBE (10 h after

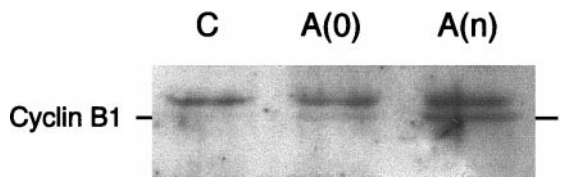

FIG. 1. Cyclin B1 levels in KE oocytes after cyclin B1-A(0) and cyclin B1-A(n) mRNA microinjection. C, control oocytes; A(0), cyclin B1-A(0) mRNA-microinjected oocytes; A(n), cyclin B1-A(n) mRNA-microinjected oocytes. Lysates of KE oocytes (collected 4 h after GVBD) were analyzed by immunoblotting using anti-cyclin B1 antibodies (all samples contained 42 oocytes).

TABLE 2
Effect of Cyclin B1 mRNA Injection on Meiosis Resumption and First Polar Body Extrusion in KE Oocytes in Cultured Medium

Microinjected mRNA	Time of PBE (mean ± SD)	% PBE (mean ± SD)	*n* (Expt)[b]	*P*[a] (time of PBE) Control	*P*[a] (time of PBE) Cyclin B1-A(n)
Control (none)	10.6 ± 0.9	82.8 ± 18.4	84 (6)		<0.0001
Cyclin B1-A(0)	9.8 ± 1.1	74.3 ± 7.6	41 (3)	<0.0001	0.003
Cyclin B1-A(30)	9.6 ± 0.8	56 ± 24.6	16 (2)	<0.0001	0.009
Cyclin B1-A(n)	8.8 ± 1.7	39.8 ± 1.4	22 (3)	<0.0001	
Ezrin	10.9 ± 1.3	77 ± 8.5	21 (2)	NS	<0.0001

Note. The oocytes were washed from dbcAMP just after the injection.
[a] Using the nonparametric Mann–Whitney test.
[b] Number of oocytes. Number of experiments in each group is given in parentheses.

GVBD) 69 ± 23.5% extruded their polar bodies and had monovalent chromosomes (Figs. 3C and 3D). The difference observed was statistically significant ($P = 0.008$). This experiment suggests that the M I arrest induced by cyclin B1 overexpression is likely due to the large store of cyclin B1 present in the oocyte. The cyclin degradation machinery would be unable to degrade enough cyclin B1 to allow a sufficient drop in MPF activity and thus PBE.

To confirm the incapacity of cyclin B1 to be degraded at a sufficient level to allow the exit of the first M phase, we microinjected a human cyclin B1–GFP mRNA (Clute and Pines, 1999; Hagting *et al.*, 1998) in GV mouse oocytes to follow the synthesis and degradation of this protein during maturation. We chose Swiss mouse oocytes because their viability is not impaired by UV illumination. First we checked that the effects on maturation induced by microinjection of the human cyclin B1–GFP mRNA were identical to those induced by the mouse cyclin B1 mRNA: oocytes microinjected with human cyclin B1–GFP mRNA underwent GVBD in dbcAMP (25% in the first hour); once released from the inhibitor, only part of them extruded their first polar bodies (54% vs 83% for the noninjected oocytes). We concluded that human cyclin B1–GFP could be used to follow the metabolism of cyclin B1 in mouse oocyte.

The fluorescence signal of the cyclin B1–GFP in oocytes that extruded their polar bodies increased during the first 5 h until it reached a plateau (Figs. 4A and 4B). Then the fluorescence signal dropped to 20% during the following 2 h. The polar body was extruded 20 min before the fluorescence signal reached its minimum. The signal increased again to reach a stable level during the M II arrest, during which equilibrium between cyclin synthesis and degradation takes place (Kubiak *et al.*, 1993).

In oocytes that did not extrude the first polar body (Fig. 4C), the cyclin B1–GFP behavior was identical during the 6 first h, but its degradation was less efficient (40% slower rate of degradation) and less complete (40% of the maximum amount left versus 20%) than in the oocytes that extruded their polar bodies. These observations suggest to us that the cyclin degradation can only take place during a given period of time. If at the end of this period, not enough cyclin B1 has been degraded, oocytes are unable to exit from the first meiotic M phase.

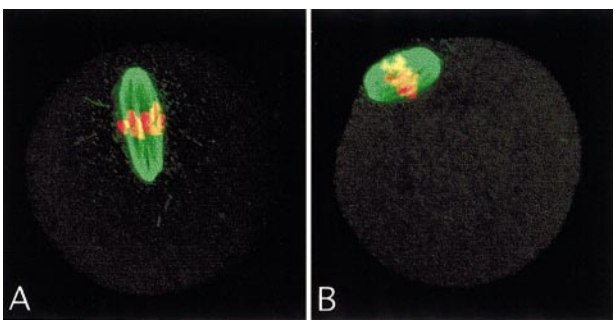

FIG. 2. Organization of microtubules and chromosomes in KE oocytes after cyclin B1-A(n) mRNA injection. Oocytes were fixed 8 h after PBE in controls. (A) Uninjected oocytes were blocked in M II. (B) Cyclin B1-A(n) mRNA-microinjected oocytes were blocked in M I with no segregation of the homolog chromosomes. Microtubules are shown in green and chromosomes in red.

Microinjection of Cyclin B1 Antisense Speeds Up PBE and Inhibits Entry into M II

In an attempt to inhibit cyclin B1 synthesis in mouse oocytes, we microinjected a mouse cyclin B1 antisense mRNA into the cytoplasm of CBA/Kw oocytes at the GV stage. The microinjected oocytes underwent GVBD normally (Table 3) and extruded their first polar bodies like the control oocytes (Table 3), although PBE was slightly accelerated (6.44 ± 0.1 h after GVBD for the cyclin B1 antisense vs 7.67 ± 0.98 h for the controls; $P < 0.0001$; Table 4). However, in contrast to the control oocytes that were arrested in M II, the microinjected oocytes entered interphase as shown by the formation of a pronucleus 3–4 h after PBE (Fig. 5) in a dose-dependant manner (Fig. 6).

When MPF activity was assessed in microinjected oo-

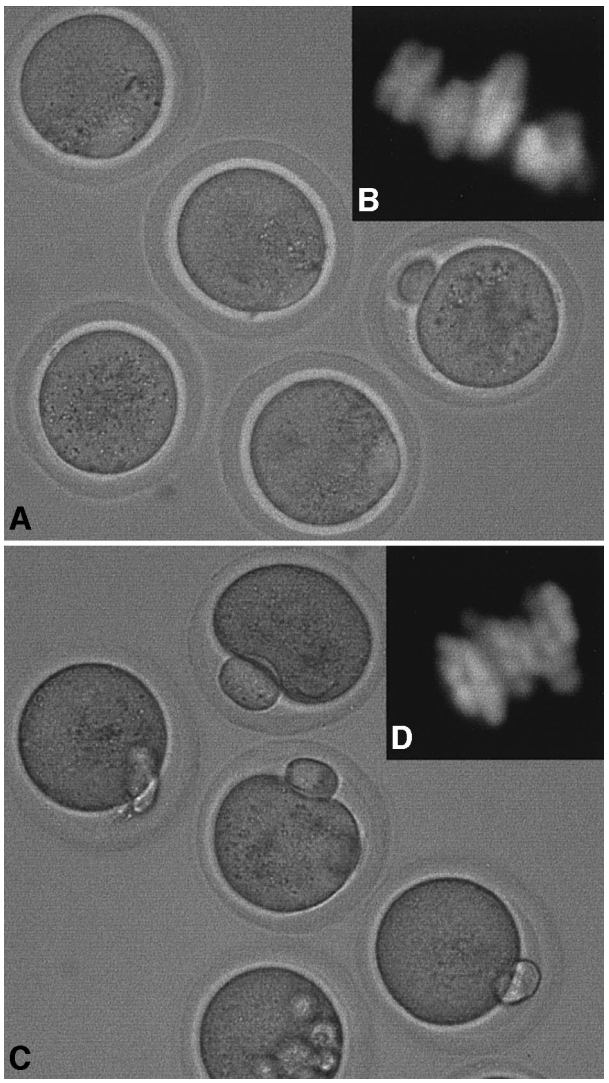

FIG. 3. Effect of ethanol treatment on M I blocked oocytes. Immature oocytes from KE strains were microinjected with cyclin B1-A(n), kept for 15 h in dbcAMP, and then washed and cultured in M2 medium. Twelve hours after GVBD the oocytes that did not extrude their first polar bodies were treated with freshly prepared 8% ethanol (C, D) for 6.5 min. Then alcohol was carefully removed by washing oocytes in M2 medium. Thirty minutes later, the first polar bodies were extruded (A, B) in non-ethanol-treated oocytes. Oocytes were fixed 4 h after ethanol treatment. The chromosomes were stained with propidium iodine (B, D).

cytes, we observed that the rise in H1 kinase activity during the first meiotic M phase was similar in microinjected and control oocytes (Fig. 7A), while it was not reactivated after PBE in microinjected oocytes (Fig. 7B). This suggested that cyclin B was synthesized during the first meiotic M phase despite the presence of the antisense. However, the antisense was able to abolish cyclin B1 synthesis at the end of the first meiotic M phase, since MPF was not reactivated. This was confirmed when the amount of cyclin B1 present in the oocytes after cyclin B1 antisense microinjection was probed by immunoblotting 6 h after GVBD (just before PBE) and the amount of cyclin B1 had already dropped to a low level in antisense-microinjected oocytes (Fig. 8A). When degradation was inhibited by nocodazole a similar amount of cyclin B1 was observed in both groups (Fig. 8B). Taking into account the low sensitivity of this assay, this may suggest that the cyclin B1 antisense had little effect on cyclin B1 synthesis during M I but was able to speed up degradation, likely through inhibition of cyclin B1 synthesis.

This lack of effect of the cyclin B1 antisense during the first meiotic M phase could be due to the synthesis of another cyclin B, cyclin B2, that could compensate for the absence of cyclin B1 synthesis. When oocytes were microinjected with a mouse cyclin B2 antisense mRNA, they underwent GVBD and were blocked in M II after PBE as controls (Table 4 and Fig. 6), suggesting that cyclin B2 was not required for PBE and MPF reactivation. Finally to check whether the synthesis of one cyclin was able to compensate for the inhibition of the synthesis of the other cyclin was able to compensate for the inhibition of the synthesis of the other cyclin, we microinjected both cyclin B1 and B2 antisenses. GVBD and PBE took place normally and the microinjected oocytes entered interphase, similar to those injected with the cyclin B1 antisense alone (Table 3).

These observations suggest that the cyclin B1 antisense mRNA has no or little effect on cyclin B1 synthesis during the first part of meiotic maturation, although it speeds up PBE and blocks the MPF reactivation required for entry into the second meiotic M phase.

The acceleration of PBE could be due to the absence of cyclin synthesis that normally takes place during the MI/MII transition. The synthesis of cyclin B that occurs simultaneously with its degradation would delay the time at which MPF activity drops to a level low enough to allow the metaphase/anaphase transition.

To test this hypothesis, we cultured CBA/KW oocytes in puromycin to block protein synthesis (including cyclin B synthesis). When 10 μg/ml puromycin was added 6 h after GVBD (about 1.5 h before PBE) the first polar body was extruded 20 min earlier than in controls (7.12 ± 0.38 ($n = 41$) for puromycin vs 7.41 ± 0.46 ($n = 50$) for controls; $P < 0.002$). This reinforces the hypothesis that inhibition of cyclin B1 synthesis at the end of the first meiotic M phase accelerates polar body extrusion.

Cyclin B1 mRNAs Are Protected Until the End of the First Meiotic M Phase

In the previous experiments, the cyclin B1 antisense seems to be able to inhibit cyclin synthesis only at the end of the first meiotic M phase, suggesting that it is not able to interfere with the cyclin B1 mRNA before that time. However, a few possible technical problems could be responsible for this effect.

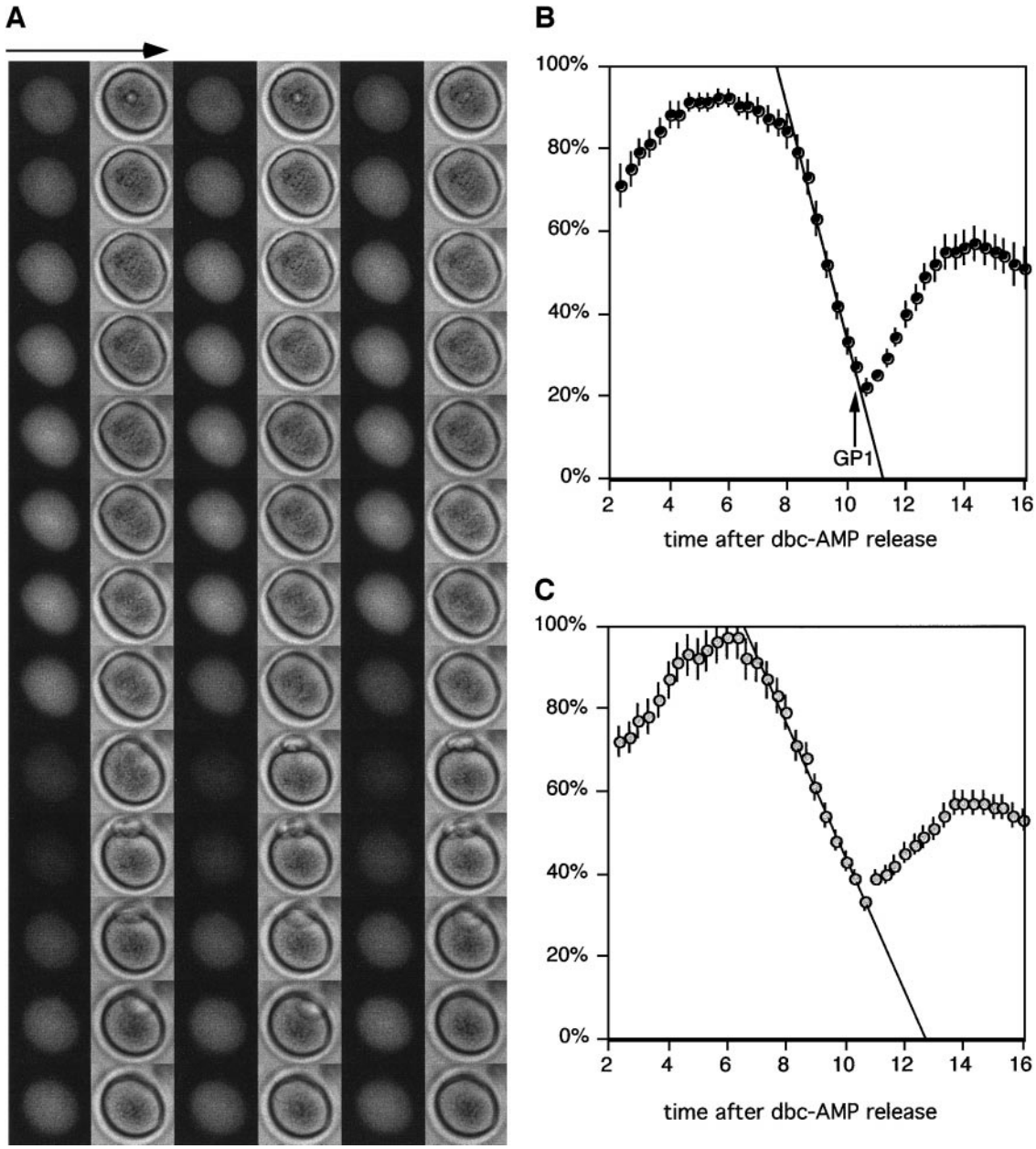

FIG. 4. Live imaging of cyclin B1–GFP in maturing swiss mouse oocytes. (A) Fluorescence and transmission images of an oocyte microinjected with cyclin B1–GFP cultured on the microscope stage throughout meiotic maturation. Images were taken every 20 min. The order of the panels is from left to right and from top to bottom. (B, C) Semiquantitative analysis of the fluorescence signal during meiotic maturation. The fluorescence was measured every 20 min and the levels of fluorescence were normalized to the maximum value for each oocyte. Data (mean ± SD) from 16 oocytes that extruded their first polar bodies (B) and 13 oocytes that did not (C) were used to plot the graphs. Oocytes from both groups were observed simultaneously under the videomicroscope. Oocytes were washed just after the microinjection.

First, a slow diffusion of the antisense in the oocyte cytoplasm could delay the interaction between the antisense and the mRNA. When we allowed the antisense to diffuse in GV-arrested oocytes before releasing the dbcAMP block for various periods of time (2, 4, and 15 h), there was no difference in the effect of the antisense: microinjected oocytes extruded their polar bodies about 1.25 h before the controls and then formed pronuclei (not shown).

TABLE 3

Effect of Cyclin B1 and B2 Antisense mRNAs on Meiotic Maturation in CBA/Kw Oocytes

Microinjected antisense mRNA	% GVBD (mean ± SD)	% PBE (mean ± SD)	% Interphase (mean ± SD)	*n* (Expt)[a]
Control (none)	85 ± 10.4	87 ± 10.1	0	315 (9)
Cyclin B1	64 ± 16.6	72 ± 14.3	85.5 ± 12.9	109 (4)
Cyclin B2	81 ± 7.8	79 ± 11.9	0	33 (3)
Cyclin B1 + B2	69.5 ± 9.9	90 ± 14.4	73.8 ± 19.9	21 (2)

Note. The CBA/Kw oocytes were injected at the GV stage with 5 A.U. of antisense and were washed from dbcAMP medium 4–5 h later.

[a] Number of oocytes. Number of experiments in each group is given in parentheses.

Second, the large size (1.5 kb) of the full-length cyclin B1 antisense could make the hybridization of the antisense with the endogenous cyclin B1 mRNA difficult. Therefore, we synthesized antisenses directed against three regions of the cyclin B1 mRNA: The 5′ region (0.4 kb), part of the coding region (0.4 kb), and the 3′ region (0.5 and 0.2 kb) of the cyclin B1 mRNA (Fig. 9A). All these shorter antisense mRNAs had similar effects: the microinjected oocytes extruded their polar bodies and formed pronuclei, as did the oocytes injected with the full-length antisense (Fig. 9B).

Third, the site of injection (cytoplasm or nucleus) could modulate the effects of the antisense mRNAs. Microinjection of two different antisense mRNAs (1.5 kb (full length) and 0.5 kb 3′ region) in the nucleus of CBA/Kw oocytes gave the same phenotype as the oocytes microinjected in the cytoplasm (not shown).

Finally, to confirm that the absence of inhibition of cyclin B1 translation during the first part of the first meiotic M phase is due to a specific masking of the cyclin

TABLE 4

Effect of Cyclin B1 and B2 Antisense RNA Injection on the Timing of GVBD and PBE in CBA/Kw Oocytes

Microinjected antisense RNA	Time of GVBD		Time of PBE (hours after GVBD)	
	mean ± SD	*n* (Expt)[a]	mean ± SD	*n* (Expt)[a]
Control (none)	1.8 ± 0.6	132 (5)	7.8 ± 1	92 (4)
Cyclin B1	1.8 ± 0.5	51 (2)	6.4 ± 0.7	49 (2)
Cyclin B2	1.7 ± 0.5	27 (2)	7.1 ± 0.8	23 (2)

Note. The CBA/Kw oocytes were injected at the GV stage with 5 A.U. of antisense and were washed 4–5 h later.

[a] Number of oocytes. Number of experiments in each group is given in parentheses.

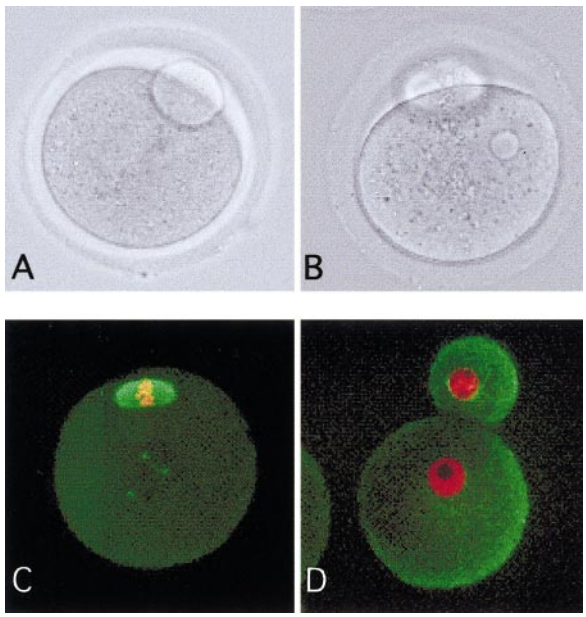

FIG. 5. CBA/Kw oocytes were microinjected with 5 a.u. of cyclin B1 antisense RNA and observed 4 h after PBE. Control oocytes were arrested in M II (A, C) and cyclin B1 antisense RNA-microinjected oocytes were in interphase with a pronucleus (B, D). Transmission (A, B) and confocal images (C, D). Oocytes used for transmission and immunofluorescence were different. Microtubules are shown in green and chromosomes in red.

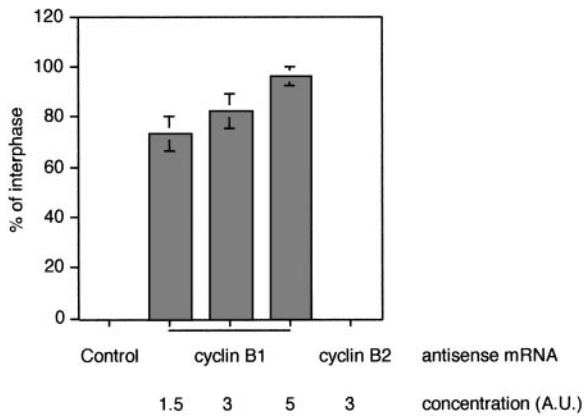

FIG. 6. Effect of cyclin B1 antisense RNA concentration on first PB extrusion and entry into the second meiotic M phase. The CBA/Kw oocytes were microinjected at the GV stage with different concentrations of antisense RNA (1.5–5 A.U.) and were washed from dbcAMP 4–5 h later. The difference between the groups of oocytes microinjected with 1.5 and 5 A.U. of cyclin B1 antisense RNA is statistically significant ($P = 0.02$ using the unpaired Welch t test). The differences between the groups of oocytes microinjected with 1.5 and 3 A.U. or 3 and 5 A.U. of cyclin B1 antisense RNA are statistically not significant ($P = 0.31$ and $P = 0.14$, respectively).

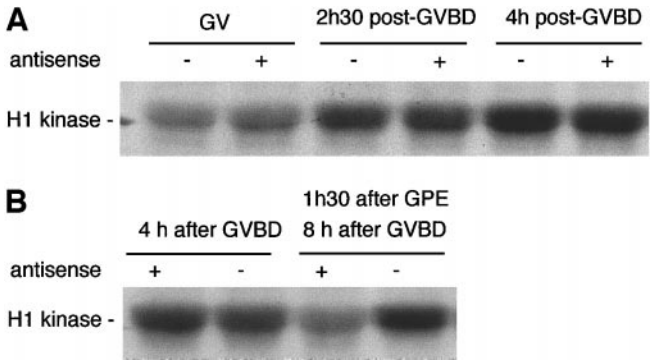

FIG. 7. Effect of cyclin B1 antisense mRNA microinjection (5 A.U.) on H1 kinase activity during the first 5 h of meiotic maturation (A) and after polar body extrusion (B) in CBA/Kw oocytes. Ten oocytes per sample were used for the H1 kinase assay.

B1 mRNA, and not to a technical problem, another mRNA whose translation has to be inhibited almost completely to show a phenotype in mouse oocytes was used. The analysis of mutant *Mos*$^{-/-}$ mice has shown that MOS is not required for GVBD and first PBE, but is absolutely required for the arrest in metaphase II (Colledge *et al.*, 1994; Verlhac *et al.*, 1996). The synthesis of this protein has to be almost completely inhibited from the beginning of meiotic maturation in order to avoid the activation of the MOS/ . . . /MAP kinase (MAPK) pathway that is responsible for the metaphase arrest: MAPK activation takes place 2 h after GVBD, when only about 10% of the total amount of MOS is synthesized (Verlhac *et al.*, 1996). The extrusion of the second polar body takes place in 75% of *Mos*$^{-/-}$ oocytes (Verlhac *et al.*, 1996). When we microinjected the full-length *Mos* antisense mRNA, we observed 32% of second polar body extrusion (Table 5). Thus, injection of the *Mos* antisense was able to block almost completely the expression of the MOS protein in one third of the oocytes. In addition, to get this effect, inhibition of MOS synthesis had to start from the beginning of the first meiotic M phase.

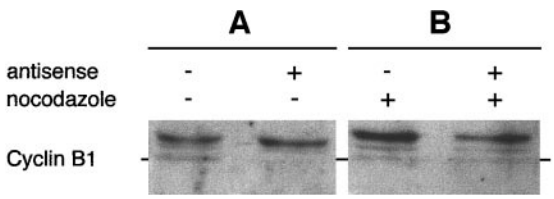

FIG. 8. Cyclin B1 levels in CBA oocytes microinjected with cyclin B1 antisense RNA 6 h after GVBD. In (B), oocytes were incubated in 10 μg/ml nocodazole starting 5 h after GVBD. Lysates were analyzed by immunoblotting using an anti-cyclin B1 antibody. Groups of 80 (A) or 60 (B) oocytes.

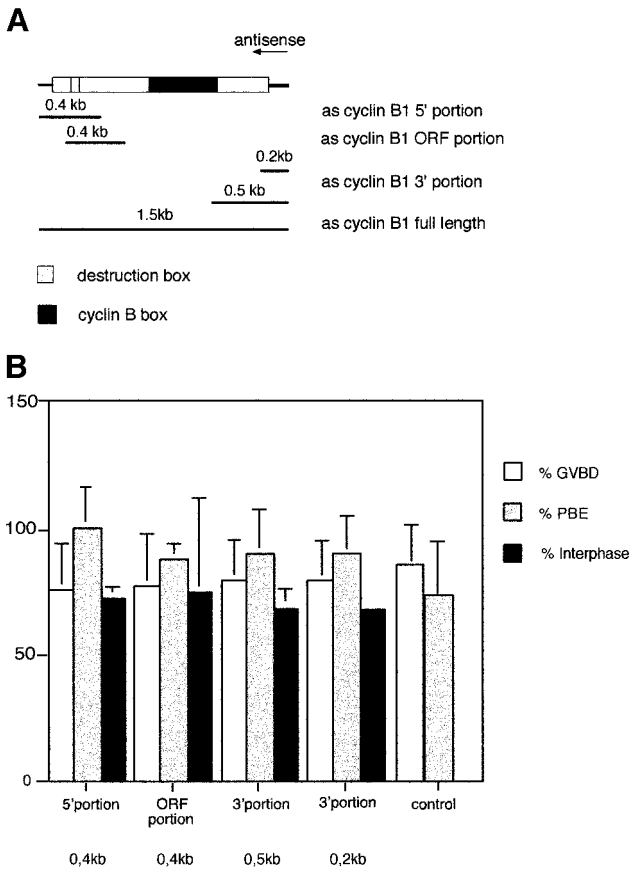

FIG. 9. (A) Schematic representation of the full-length cyclin B1 cDNA and different cyclin B1 antisense RNAs produced. (B) Effect of the microinjection of the different cyclin B1 antisense RNAs (5 A.U.) on meiotic maturation in CBA/Kw oocytes. The oocytes were injected at the GV stage and washed from dbcAMP 4–5 h later.

All these results suggest that, in contrast to the *Mos* mRNA, the cyclin B1 mRNA is protected until the end of the first meiotic M phase in the mouse oocyte.

TABLE 5

Effect of c-*Mos* Antisense Injection on Meiotic Maturation in Swiss Oocytes

Microinjected antisense RNA	% GVBD (mean ± SD)	% PBE 1 (mean ± SD)	% PBE 2 (mean ± SD)	*n* (Expt)[a]
Control (none)	95 ± 5	93.6 ± 2	0	62 (2)
c-*Mos*	94.4 ± 5.5	85.7 ± 2	32 ± 3	55 (2)

Note. Oocytes were microinjected at the GV stage and washed from dbcAMP 4 h later.

[a] Number of oocytes. Number of experiments in each group is given in parentheses.

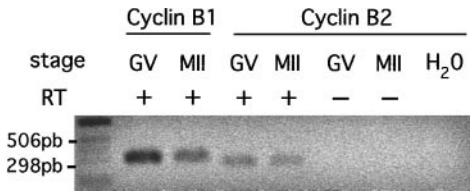

FIG. 10. Expression of the cyclin B1 and B2 mRNAs during meiotic maturation in CBA/Kw oocytes. The products of polymerase chain reaction on reverse-transcribed mRNAs (RT+) were analyzed on a 2% agarose gel. RT− corresponds to controls where the reverse transcription step was omitted.

Cyclin B2 and Mouse Meiotic Maturation

Although cyclin B2 is not required for mouse development and meiotic maturation (Brandeis *et al.*, 1998), the cyclin B2 mRNA is present in the immature oocytes (Chapman and Wolgemuth, 1993). We confirmed by RT-PCR that the cyclin B2 mRNA was present in GV and in M II oocytes (Fig. 10). To test for a possible role of cyclin B2 in meiotic maturation of the mouse oocyte, we microinjected cyclin B2-A(n) mRNA into KE oocytes. The oocytes resumed meiosis and extruded their first PB as the controls (Table 6). In the presence of dbcAMP, cyclin B2-A(n) mRNA induced GVBD more efficiently than cyclin B1-A(n) mRNA (67% ± 10 versus 36.8% ± 6.2). Moreover, 80% of the oocytes broke down their GV during the first 2.5 h following microinjection versus 36.8% for the oocytes microinjected with cyclin B1-A(n) mRNA. However, the oocytes microinjected with cyclin B2-A(n) mRNA that resumed meiosis in dbcAMP extruded the first polar bodies almost normally (64.8% ± 0.6) in contrast to the oocytes microinjected with cyclin B1-A(n) mRNA which were mostly blocked in M I (16.7% ± 4.0 of PBE; Table 7). Thus overexpression of cyclin B2 very efficiently overcomes the dbcAMP block but does not induce an M I arrest.

DISCUSSION

In this work, we attempted to modify the level of cyclin B during the maturation of the mouse oocyte by overexpressing or inhibiting the synthesis of cyclin B1 in KE and in CBA/Kw oocytes, respectively. We observed that overexpression of cyclin B1 had many consequences on meiotic maturation. First the microinjected oocytes were able to bypass the dbcAMP-induced GV block. Second, cyclin B1-A(n) mRNA-microinjected oocytes presented two phenotypes: most (60%) were blocked in MI while the others extruded their first polar bodies in advance.

The use of antisense mRNAs to inhibit cyclin B1 synthesis suggested that the endogenous cyclin B1 mRNAs were protected until the end of the first meiotic M phase. Nevertheless, cyclin B1 synthesis was inhibited at the metaphase/anaphase transition since polar body extrusion was accelerated and oocytes were not able to reactivate MPF to enter into the second meiotic M phase.

Control of GVBD

In mouse oocytes, new protein synthesis is not necessary for GVBD (Clarke and Masui, 1983; Stern and Wassarman, 1973) although cyclin B is synthesized at a low level in mouse oocytes at the GV stage (Hampl and Eppig, 1995b; Winston, 1997). It is thought that GVBD in mouse oocytes is due to the dephosphorylation of a small pool of pre-MPF (Choi *et al.*, 1991, 1992).

Cyclin B1 mRNA-microinjected oocytes were able to bypass the dbcAMP-induced GV block. Two hypotheses can explain how cyclin B1 translation is able to bypass this block. First, the overexpression of cyclin B1 could lead to the formation of MPF that is not inactivated by phosphorylation. Second, it could activate the cdc25 phosphatase that dephosphorylates the pool of pre-MPF. We favor the first hypothesis since overexpression of cyclin B1 overcomes the orthovanadate-induced GV block that is thought to be due to inhibition of cdc25 phosphatase (64% of oocytes injected with cyclin B1-A(n) mRNA (n = 41) underwent GVBD in the presence of 2 mM orthovanadate while only 7% (n = 44) of the control oocytes resumed meiosis) (Choi *et al.*, 1992).

Our results suggest also that the size of the poly(A) tail is responsible for the level of cyclin B1 translation since only the mRNAs with a long poly(A) tail were able to induce the resumption of meiosis in the presence of dbcAMP. These results are in agreement with those of Tay *et al.* (2000)

TABLE 6

Effect of Cyclin B2-A(n) mRNA Injection on Meiosis Resumption and First Polar Body Extrusion in KE Oocytes

Microinjected mRNA	% GVBD (mean ± SD)	% PBE (mean ± SD)	P^a GVBD	P^a PBE	n (Expt)^b
Control	82.8 ± 10.9	62.3 ± 17.7	0.07 (NS)	0.03 (NS)	109 (6)
Cyclin B2-A(n)	93 ± 9.8	94.3 ± 8			114 (6)

Note. Oocytes were microinjected at the GV stage and immediately washed from dbcAMP.

[a] Using the nonparametric Mann–Whitney test.

[b] Number of oocytes. Number of experiments in each group is given in parentheses.

TABLE 7
Effect of Cyclin B2-A(n) mRNA Injection on Meiosis Resumption and First Polar Body Extrusion in KE Oocytes in the Presence of dbcAMP

Microinjected mRNA	% GVBD (mean ± SD)	% PBE (mean ± SD)	*n* (Expt)[a]
Control (none)	0	0	80 (4)
Cyclin B2-A(n)	67 ± 10	64.8 ± 9.8	83 (4)
Cyclin B1-A(n)	36.8 ± 6.3	16.7 ± 3.8	277 (6)

[a] Number of oocytes. Number of experiments in each group is given in parentheses.

showing that polyadenylation of cyclin B1 mRNA is required for translation activation during mouse oocyte maturation. For many mRNAs, the addition of A residues promotes translational activation. For example, polyadenylation of *Mos* mRNA in *Xenopus* and in mouse oocytes is required for meiotic maturation (Gebauer *et al.,* 1994; Sheets *et al.,* 1994). Oocyte injection studies have demonstrated that the activation of tissue-type plasminogen activator in the mouse is also regulated by the number of 3′ adenylated residues (Huarte *et al.,* 1992; Vassalli *et al.,* 1989).

Progression of Meiotic Maturation and Exit from the First M Phase

In the absence of protein synthesis, GVBD takes place but the oocyte does not progress through meiosis I: the meiotic spindle does not form and MPF activity drops in 3–4 h to a low level (Polanski *et al.,* 1998; Clarke and Masui, 1983). Earlier studies have shown that, during the first meiotic M phase, the level of cyclin B1 synthesis in CBA/Kw oocytes is higher than that in KE oocytes and that MPF activation is slower in KE oocytes (Polanski *et al.,* 1998). All these differences are responsible for the delayed PBE in KE oocytes since the microinjection of polyadenylated cyclin B1 mRNA in KE oocytes increases the level of MPF activity and speeds up PBE (Polanski *et al.,* 1998).

In the experiments described above, we observed that the acceleration of PBE is correlated with the size of the poly(A) tail, suggesting that the longer the poly(A) tail, the more cyclin B1 synthesis. Recent studies have shown that the length of the poly(A) tail of cyclin B1 mRNA increases progressively during meiotic maturation, moderately between GV to M I (100 nucleotides) and more importantly (250 nucleotides) between M I and M II (Tay *et al.,* 2000). This increase coincides with the progressive rise of cyclin B1 synthesis during meiotic maturation (Hampl and Eppig, 1995a; Winston, 1997). We conclude that the level of translation of the cyclin B1 through the length of the poly(A) tail controls the timing of the first M phase.

Another interesting observation was made in oocytes that expressed very high levels of cyclin B1 (those able to overcome the dbcAMP block): most of them never extruded their first PB even 12 h after the normal time of PBE. This could be due to the inability of these oocytes to degrade enough cyclin B1 to reach the threshold of MPF activity required for exit from M phase and to the fact that degradation can only occur during a given period of time once it has started. When we followed the synthesis and the degradation of cyclin B1–GFP in oocytes that extruded their first polar bodies, we observed that cyclin B1–GFP started to be degraded 2 h before PBE. In oocytes that did not extrude their first polar bodies, we observed that degradation of cyclin B1–GFP was going on during a limited period of time (about 4 h) and that the minimum level of cyclin B1 observed in controls was not reached. Then, in both groups of oocytes, when cyclin degradation was switch off, an increase in cyclin B1–GFP was observed. This was followed by a steady state that most likely corresponds to the equilibrium between synthesis and degradation that was previously described in Kubiak *et al.* (1993).

The Protection of the Cyclin B1 mRNA to the Antisense

To test the effect of inhibition of cyclin B1 synthesis on maturation we used the antisense technique. Surprisingly the CBA/Kw oocytes microinjected with cyclin B1 antisense mRNA progressed trough the first meiotic M phase normally and extruded their first polar bodies although they did so earlier than the control oocytes. However, in contrast to the control oocytes that entered into the second meiotic M phase, the microinjected oocytes entered into interphase with a low MPF activity after PBE. Since we know that cyclin B synthesis is required for the progression of the first meiotic M phase, our observation suggests that antisense mRNA is active only at the end of the first meiotic M phase.

An antisense against the 5′ region of the globin mRNA in frog oocyte is sufficient to inhibit its synthesis (Melton, 1985), while an antisense RNA directed against the 3′ noncoding region of the tPA (tissue-type plasminogen activator) prevents the activation of this dormant mRNA in mouse oocytes (Strickland *et al.,* 1988). In our experiments, the microinjection of antisense mRNA with different sizes and directed against different regions of the cyclin B1 mRNA had the same effect as the full-length antisense. Thus, this late accessibility of the endogenous cyclin B1 mRNA to the antisense was not due to the long size of the antisense that could have difficulties hybridizing with the endogenous cyclin B1 mRNA. This suggests a masking effect that protects the mRNA until the end of the first meiotic M phase in the mouse oocyte. Tay *et al.* demonstrated that the mouse cyclin B1–CPEB (cytoplasmic polyadenylation element binding protein) binds the ACE (adenylation cytoplasmic element) at the GV stage and repressed cyclin B1 translation (Tay *et al.,* 2000). CPEB is phosphorylated during the first meiotic M phase. This could modify the accessibility of cyclin B1 mRNA. We

propose that at the end of the first meiotic M phase, cyclin B1 mRNA becomes accessible to the antisense leading to an inhibition of cyclin B1 synthesis. This would speed up cyclin B1 degradation (because no cyclin B1 synthesis would occur at that time) and lead to a slight acceleration of PBE.

What Is the Role of Cyclin B2 during Meiotic Maturation?

Very little is known about the role of the different cyclin Bs during meiotic maturation in vertebrates and the presence of different cyclin Bs raises a question. In *Rana japonica* cyclin B1 or B2 is necessary and sufficient to induce GVBD (Ihara *et al.*, 1998). In *Xenopus* oocytes, these two cyclins seem to be expressed and complexed with cdks differently during meiotic maturation. They are destroyed with different kinetics in anaphase: cyclin B2 is degraded earlier than cyclin B1 (Kobayashi *et al.*, 1991; Minshull *et al.*, 1989). Cyclin B1 and B2 are coexpressed in the majority of the dividing cell although the intracellular distribution of the two cyclin Bs is different in human cultured cells: cyclin B1 localizes to the microtubules and cyclin B2 to the golgi apparatus in interphase. Cyclin B1, but not cyclin B2, enters in the nucleus at the G2/M transition and is found on the spindle at metaphase (Gallant and Nigg, 1992; Jackman *et al.*, 1995; Ookata *et al.*, 1992; Pines and Hunter, 1991). All these differences suggest different roles for these two cyclin Bs, both in meiosis and in mitosis.

In mouse oocytes, both cyclin B1 and B2 transcripts are present during meiotic maturation (Chapman and Wolgemuth, 1992, 1993, and our work). We were able to detect the presence of cyclin B2 during the M II arrest (data not shown), although the sensitivity of the assay was very poor since more than 400 oocytes were required to get a signal, making a study of the expression of this protein during meiotic maturation unworkable. However, mice lacking functional cyclin B2 develop normally and are fertile whereas the nullizigous B1 mice are embryonic lethal (Brandeis *et al.*, 1998). This indicates that meiotic maturation can take place in the absence of cyclin B2 and would explain the lack of effects of the cyclin B2 antisense.

However, the overexpression of cyclin B2 gave us some interesting results: cyclin B2 was more efficient than cyclin B1 in inducing GVBD in the presence of dbcAMP but was not able to block polar body extrusion. This argues also for different roles of cyclin B1 and B2 during meiosis. Recent studies in *Xenopus* oocytes suggested that the correct localization of cyclin B2, but not that of cyclin B1, is essential for bipolar spindle formation (Yoshitome *et al.*, 1998). However, in mouse oocytes, cyclin B2 does not seem to play a role in the setting up of a bipolar structure since maturation is normal in oocytes from mice lacking cyclin B2 (Brandeis *et al.*, 1998), which implies that a functional spindle was formed. Thus it is possible that cyclin B2 plays a specific role in the entry into meiotic M phase (although, it could be functionally replaced by cyclin B1).

Taken together, our data demonstrate that the rates of cyclin B synthesis and degradation determine the timing of the major events taking place during meiotic maturation of the mouse oocyte. Although some progress has been made in the characterization of the molecular mechanisms controlling cyclin B metabolism during meiotic maturation of the mouse oocyte (Barkoff *et al.*, 2000; de Moor and Richter, 1999; Kubiak *et al.*, 1993; Polanski *et al.*, 1998; Tay *et al.*, 2000), more work is still required to fully understand them.

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