Xenopus Oocyte Maturation Does Not Require New Cyclin Synthesis

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Abstract. Progesterone induces fully grown, stage VI, *Xenopus* oocytes to pass through meiosis I and arrest in metaphase of meiosis II. Protein synthesis is required twice in this process: in order to activate maturation promoting factor (MPF) which induces meiosis I, and then again after the completion of meiosis I to reactivate MPF in order to induce meiosis II. We have used antisense oligonucleotides to destroy maternal stores of cyclin mRNAs, and demonstrate that new cyclin synthesis is not required for entry into either meiosis I or II. This finding is consistent with the demonstration that stage VI oocytes contain a store of B-type cyclin polypeptides (Kobayashi, H., J. Minshull, C. Ford, R. Golsteyn, R. Poon, and T. Hunt. 1991. *J. Cell Biol.* 114:755–765). Although ~70% of cyclin B2 is destroyed at first meiosis, the surviving fraction, together with a larger pool of surviving cyclin B1, must be sufficient to allow the reactivation of MPF and induce entry into second meiotic metaphase. Since stage VI oocytes do not contain any cyclin A, our results show that cyclin A is not required for meiosis in *Xenopus*. We discuss the possible nature of the proteins whose synthesis is required to induce meiosis I and II.

We have investigated the role of cyclin synthesis in the meiotic divisions of *Xenopus* oocytes. Fully grown, stage VI *Xenopus* oocytes are arrested late in G2 and are induced to enter meiosis and mature into unfertilized eggs by progesterone (see Fig. 1). Progesterone triggers a complex set of reactions that lead to the activation of maturation promoting factor (MPF),[1] the protein kinase composed of $p34^{cdc2}$ and cyclin that induces the G2→M transition. The first activation of MPF in stage VI oocytes induces meiosis I. A transient decline in MPF activity marks the transition between meiosis I and II, and MPF activity reappears again at the onset of meiosis II (Gerhart et al., 1984). MPF levels then remain high, causing the cell cycle to arrest in metaphase of meiosis II until fertilization. Protein synthesis is required twice during maturation: to induce meiosis I and, after the completion of meiosis I, to induce meiosis II (Gerhart et al., 1984; Wasserman and Masui, 1975). The observations that microinjection of cyclin mRNA can induce oocyte maturation (Swenson et al., 1986; Pines and Hunt, 1987) and that cyclin must be synthesized after fertilization in order to induce mitosis (Minshull et al., 1989; Murray and Kirschner, 1989) suggested that cyclin synthesis was required for the induction of meiosis by progesterone.

Previous work has provided strong evidence that synthesis of c-*mos* is required for oocyte maturation (Sagata et al., 1988). This was based on the ability of antisense oligonucleotides directed against *Xenopus* c-*mos* to block maturation, as well as on the maturation-promoting activity of injected *mos* mRNA (Freeman et al., 1990; Sagata et al., 1989). The accompanying paper shows that unlike c-*mos*, substantial stores of cyclin already exist in stage VI oocytes. In the accompanying paper, we show that stage VI *Xenopus* oocytes contain a store of ~200 pg cyclin B2 polypeptide, lower amounts of cyclin B1, and no detectable cyclin A. We found that the synthesis of all three cyclins was strongly stimulated by treatment with progesterone.

We have investigated the relative contribution of maternal cyclin and newly synthesized cyclin in progesterone-induced maturation. We ablated all three endogenous cyclin mRNAs (Minshull et al., 1989, 1990) by microinjecting antisense oligodeoxyribonucleotides into immature oocytes, and then checked the ability of the oocytes to mature in response to progesterone. We show here that oocytes in which all cyclin mRNA had been degraded were still able to mature in response to progesterone. This suggests that the stores of cyclin B1 and B2 proteins that are present in *Xenopus* oocytes are sufficient to permit the activation of the $p34^{cdc2}$ kinase, and that enough cyclin survives destruction at the metaphase to anaphase transition of meiosis I to permit the cells to enter meiosis II and stably arrest at that phase of the cell cycle. Finally, our data suggest that cyclin A is not required for oocyte maturation.

Materials and Methods

Oocyte Preparation

Oocytes were surgically collected from primed frogs, manually defolliculated, microinjected, incubated in modified, modified Ringer's solution

1. *Abbreviations used in this paper*: CSF, cytostatic factor; GVBD, germinal vesicle breakdown; MPF, maturation promoting factor; MMR, modified, modified Ringer's solution.

(MMR), and scored for germinal vesicle breakdown (GVBD) as described by Gerhart et al. (1984).

Antisense Ablation of Cyclin mRNAs in Oocytes

Oligonucleotides were dissolved in water and injected in a volume of 50 nl. The oligonucleotides used to ablate cyclin mRNAs were: anticyclin B1, 75 ng per oocyte of 5′-CCATTGGGCTTGGTGAGC-3′; anticyclin B2, 75 ng per oocyte of 5′-GGACACATCCATCGGCAC-3′; and anticyclin A, 100 ng per oocyte of 5′-CCAAACTGGGACAATC-3′. These oligonucleotides were each tested for their ability to cause destruction of their cognate mRNAs, first in a crude cell-free extract and then in intact oocytes. We used the lowest amounts that consistently led to the degradation of >95% of the cognate cyclin mRNA. All these oligonucleotides are complementary to their mRNAs within the first 100 codons, so that even if the 5′-cleavage product was stable, it would not encode a protein containing any of the sequences conserved between different cyclins. The control oligonucleotide used was 250 ng of 5′-GATTGTCCCAGTTTGG-3′, the complement of the anti-A oligonucleotide.

Oocytes were incubated for 150 min after injection to allow mRNA cutting by endogenous RNase H. Progesterone was then added to the medium to a final concentration of 10 μg/ml. Oocytes underwent GVBD between 4 and 6 h after progesterone addition and were frozen individually in liquid nitrogen and stored at −80°C until analysis.

Analysis of Cyclin mRNA and H1 Kinase Levels in Single Oocytes

Oocytes were thawed and homogenized in 20 μl of H1 kinase buffer (80 mM β-glycerophosphate, pH 7.4, 15 mM $MgCl_2$, 20 mM EGTA, 50 μM ATP, 0.1% NP-40, 15 μg/ml benzamidine, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 10 μg/ml soybean trypsin inhibitor). Half of the homogenate was pipetted into 100 μl of RNase protection buffer and 100 μl of 1:1 phenol/chloroform. RNase protection was performed as previously described (Minshull et al., 1989).

The other half of the homogenate was assayed for H1 kinase activity. After spinning for 3 min at full speed in a microfuge at 4°C, 9 μl of the supernatant (equivalent to half an oocyte) were removed and added to 1 μl of H1 kinase buffer containing 2.5 μg of histone H1 (Boehringer Mannheim Biochemicals, Indianapolis, IN) and 1.25 μCi of γ^{32}P-ATP (Amersham International, Amersham, UK). The reaction was incubated at 30°C for 10 min, stopped by the addition of 30 μl of SDS sample buffer, and 10-μl samples were analyzed on a 15% polyacrylamide gel (Anderson et al., 1973) and autoradiographed.

Maturation of Oocytes in Cycloheximide

Antisense-injected oocytes or controls were incubated in MMR containing 10 μg/ml progesterone until GVBD. Oocytes were then either transferred into MMR containing 100 μg/ml cycloheximide and incubated for 2 h, or incubated for a further 6 h, and then placed in cycloheximide for 2 h.

To follow the destruction of cyclin and loss of H1 kinase at GVBD, oocytes were incubated in MMR containing 10 μg/ml progesterone and five oocytes were sampled at the indicated times. At 120 min, 15 min before $GVBD_{50}$, the oocytes were placed into MMR with 10 μg/ml progesterone and 100 μg/ml cycloheximide. Oocytes were collected into groups that underwent GVBD within 10 min of each other, and samples of five oocytes were frozen at the times indicated after GVBD.

Analysis of Cyclin Protein and H1 Kinase Levels in Oocytes

Groups of five oocytes were homogenized in 25 μl of H1 kinase buffer containing 200 mM sucrose, 100 mM NaCl, 3 mM PMSF, but no NP-40, spun in a microfuge for 3 min, and the supernatant was divided for analysis of H1 kinase and cyclin protein. For H1 kinase assays, 2.5 μl of supernatant were added to 7.5 μl of H1 kinase buffer containing histone H1 and γ^{32}P-ATP and reactions were performed as described above. To measure cyclin protein levels, 20 μl of supernatant were added to an equal volume of 2× SDS sample buffer, and 5 μl (equivalent to half an oocyte) were loaded onto a 15% SDS polyacrylamide gel, transferred to nitrocellulose, and probed with anticyclin antibodies as previously described (Gautier et al., 1990).

Activation of Oocytes Matured In Vitro

In vitro matured oocytes were activated by injection of 50 nl of 1 mM $CaCl_2$ in 100 mM KCl, 50 mM Hepes, pH 7.0. Subsequent cortical contraction and surface contraction waves were monitored by time-lapse video. Activated oocytes for analysis of H1 kinase destruction were frozen 25 min after the calcium injection.

Results

Progesterone Induces GVBD in Xenopus Oocytes Lacking Cyclin mRNAs

The accompanying paper shows that *Xenopus* oocytes contain three different cyclin mRNAs encoding cyclins A, B1, and B2. To test the requirement for cyclin synthesis during maturation, oocytes were injected with oligonucleotides complementary to cyclin A mRNA, to cyclin B1 and B2 mRNAs, or with a mixture of these oligonucleotides complementary to all three *Xenopus* cyclins. After incubating the oocytes for 2 h to allow complete cleavage of the cyclin mRNAs by the endogenous RNase H, the oocytes were incubated with progesterone. Entry into first meiosis was assayed by the appearance of a white spot in the centre of the animal pole that indicates GVBD.

Progesterone addition induced GVBD even in oocytes that had been injected with the mixture of oligonucleotides complementary to all known cyclin mRNAs. To demonstrate that these treatments had indeed ablated cyclin mRNAs, the extent of cyclin mRNA cleavage was determined by RNase protection mapping of the RNA from individual oocytes that had undergone GVBD. Fig. 2 shows that oocytes which underwent GVBD after injection with anticyclin oligonucleotides had indeed lost their cyclin mRNAs. GVBD was observed in only ~20% of oocytes injected with antisense oligonucleotides and then treated with progesterone. We emphasize that in all of the antisense-injected oocytes that matured, all three cyclin mRNAs had been reduced to <5% of their normal levels. It could be argued that these oocytes represent a subpopulation whose maturation has a diminished or absent requirement for cyclin synthesis. However, a control oligonucleotide that did not ablate cyclin mRNAs (Fig. 2, lane *11*) also caused an 80% inhibition of GVBD, and we ascribe the inhibition of GVBD in oligonucleotide-injected oocytes to nonspecific side effects of the large quantities of single-stranded DNA that had to be injected in order to obtain complete ablation of the cyclin mRNAs (Minshull et al., 1989; Minshull and Hunt, 1986; Woolf et al., 1990; Smith et al., 1991).

H1 Kinase Is Activated in the Absence of New Cyclin Synthesis

To demonstrate that progesterone treatment had activated MPF in the oocytes whose cyclin mRNA had been ablated, we assayed histone H1 kinase activity and the integrity of cyclin mRNA in homogenates of individual oocytes that had undergone GVBD. Histone H1 is an in vitro substrate for active MPF (Arion et al., 1988; Gautier et al., 1988), so MPF levels can be assessed by measurement of H1 kinase activity. Fig. 3 shows phosphorylation of histone H1 by extracts prepared from oocytes that underwent GVBD after the ablation of all three cyclin mRNAs (Fig. 3, lanes *1–3*). All the injected oocytes we tested showed activation of H1 kinase at the time of GVBD. Uninjected oocytes also underwent GVBD in response to progesterone and had high levels of H1 kinase (Fig. 3, lane *4*). H1 kinase remained low, and GVBD did not occur

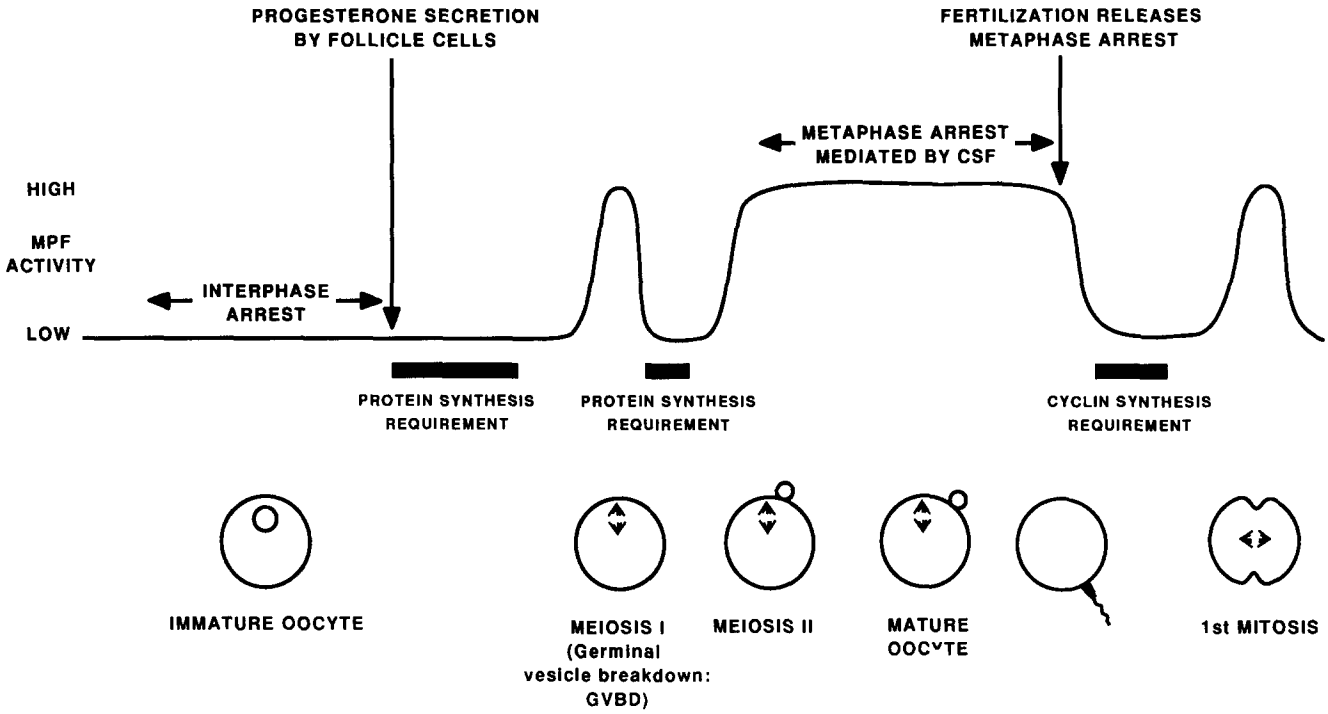

Figure 1. Protein synthesis requirements for entry into M-phase in early *Xenopus* cell divisions. A schematic view of *Xenopus* oocyte maturation and first mitoses. Levels of MPF/H1 kinase and the physiological stimuli that induce these changes are indicated.

in oocytes that were not treated with progesterone (Fig. 3, lane *5*). Similarly, there was no activation of H1 kinase in oocytes that had been injected with oligonucleotides but failed to undergo GVBD due to the nonspecific inhibition caused by large amounts of single-stranded DNA (data not shown).

Oocytes Without Cyclin mRNAs Can Still Enter Second Meiosis

The refractility of the yolk platelets makes it impossible to directly observe meiosis in living *Xenopus* oocytes. We therefore established a biochemical criterion for assessing whether oocytes had passed through meiosis I and entered meiosis II. Normally, MPF levels and H1 kinase activity both fall after meiosis I (Gerhart et al., 1984; our unpublished observations). Protein synthesis is necessary to allow a second rise in MPF and entry into meiosis II (Gerhart et al., 1984). Oocytes in meiosis I and meiosis II can thus be distinguished by their ability to maintain MPF activity after inhibiting protein synthesis. Oocytes whose protein synthesis is inhibited in meiosis I cannot maintain high levels of MPF or H1 kinase activity. In contrast, unfertilized eggs that have reached metaphase of meiosis II can maintain high levels of MPF even when protein synthesis is inhibited (Gerhart et al., 1984). Since MPF levels correlate closely with the activity of H1 kinase (Arion et al., 1988; Gautier et al., 1988), these observations suggested that oocytes in meiosis I and II could be distinguished by their ability to maintain high levels of H1 kinase activity after inhibiting protein synthesis. To arrest protein synthesis at meiosis I, we incubated oocytes in progesterone and then added cycloheximide 15 min before GVBD. The H1 kinase activity in these oocytes increased at the time of GVBD, and fell sharply soon afterwards (Fig. 3, lane *6*). However, when oocytes were allowed to reach meio-

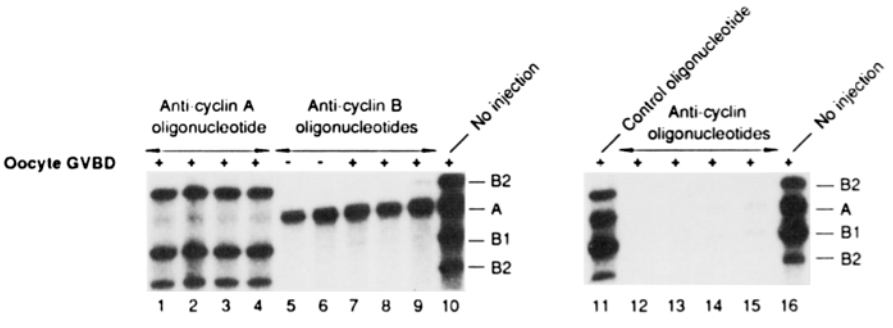

Figure 2. Oocytes mature after antisense oligonucleotide–directed ablation of cyclin mRNAs. Oocytes were injected with anticyclin oligonucleotides and treated with progesterone as described in Materials and Methods. Cyclin mRNAs present in single oocytes were analyzed by RNase protection. Positions of probes corresponding to full-length mRNAs are indicated. (lanes *(1–4)* Anticyclin A; (lanes *5–9*) anticyclins B1 and B2; (lanes *10* and *16*) uninjected oocytes; (lane *11*) control oligonucleotide; (lanes *12–15*) anticyclins A, B1, and B2. Densitometry indicated that >95% of targeted mRNAs were destroyed by the oligonucleotides used. The same degree of cutting was seen in oocytes examined immediately before addition of progesterone, indicating that RNA cleavage was complete before addition of hormone (data not shown). For all oligonucleotides the ablation of cyclin mRNAs was specific; no degradation of the other cyclins, or actin mRNA was seen.

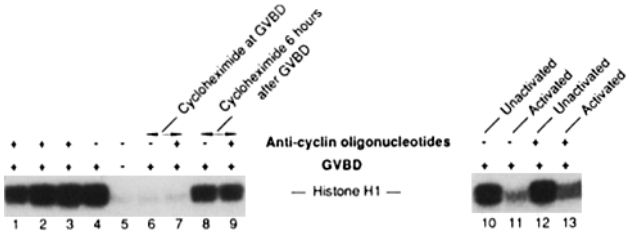

Figure 3. H1 kinase activity in oocytes after GVBD without cyclin translation. H1 kinase activity was measured in the oocytes that had undergone GVBD. Individual oocytes that had been injected with a mixture of anticyclin oligonucleotides directed against cyclin A, B1, and B2 mRNAs, were analyzed in parallel by RNase protection to confirm that the mRNAs had been cleaved. (lanes *1–3*) Oocytes that underwent GVBD after cyclin mRNA ablation (frozen at GVBD); (lane *4*) uninjected matured oocyte (frozen at GVBD); (lane *5*) uninjected oocyte not exposed to progesterone. (lanes *6–9*) Oocytes placed into cycloheximide and assayed for H1 kinase 2 h later. (lane *6*) Uninjected oocyte, cycloheximide added at GVBD; (lane *7*) cyclin mRNAs ablated, cycloheximide added at GVBD; (lane *8*) uninjected oocyte, cycloheximide added 6 h after GVBD; (lane *9*) cyclin mRNAs ablated, cycloheximide added 6 h after GVBD. (lanes *10–13*) Progesterone-matured oocytes activated with calcium 16 h after progesterone addition. (lane *10*) Control oligonucleotide, no activating injection; (lane *11*) control oligonucleotide, activated; (lane *12*) cyclin mRNAs ablated, no activating injection; (lane *13*) cyclin mRNAs ablated, activated.

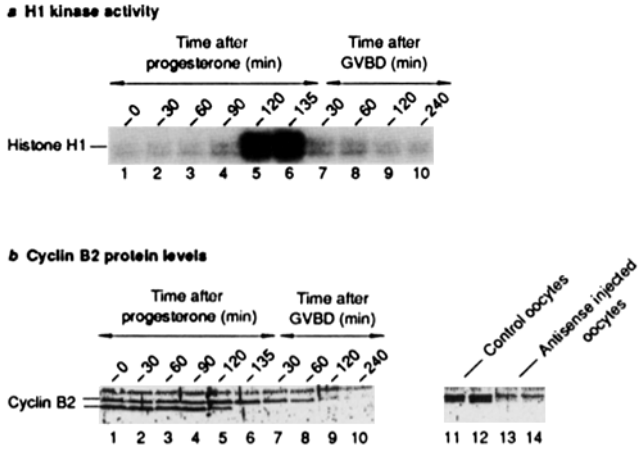

Figure 4. H1 kinase activity and cyclin B levels in oocytes treated with progesterone and cycloheximide. Oocytes treated with cycloheximide just before GVBD were analyzed for H1 kinase activity and cyclin B2 levels. Half the oocytes had "white spots" at 135 min ($GVBD_{50}$). Up to and including 135 min, only oocytes without GVBD were analyzed, while all later samples refer to oocytes that had undergone GVBD. Oocytes were taken at 0, 30, 60, 90, 120, 135 min after addition of progesterone, then at 30, 60, 120, and 240 min after GVBD. (*a*) Phosphorylation of histone H1 by oocyte extracts; (*b*) cyclin B2 levels. (lanes *11–14*) Cyclin B2 levels in individual matured oocytes 16 h after addition of progesterone, but without cycloheximide. (lanes *11* and *12*) Control oocytes; (lanes *13* and *14*) oocytes in which cyclin mRNAs were ablated.

sis II before the addition of cycloheximide (Fig. 3, lane *9*), the inhibition of further protein synthesis had no effect on the level of H1 kinase.

We asked whether oocytes could enter second meiosis after cyclin mRNA ablation and treatment with progesterone. When protein synthesis was blocked in these oocytes at the time of GVBD, they lost their H1 kinase activity within 2 h (Fig. 3, lanes *6* and *7*). By contrast, oocytes in which protein synthesis was not inhibited until 6 h after GVBD, contained H1 kinase activity and maintained it during a further 2-h incubation in cycloheximide (Fig. 3, lanes *8* and *9*), suggesting that these oocytes had entered second meiotic metaphase, even though they lacked intact cyclin mRNA.

We also tested other criteria for entry into meiosis II. Thus, mature oocytes respond to increases in the cytoplasmic calcium concentration by inactivating MPF and undergoing a cortical pigment contraction. Control oocytes that had been incubated in progesterone for 16 h showed a cortical pigment contraction and lost H1 kinase activity when they were injected with calcium, and so did oocytes that had been incubated in progesterone after ablation of all three cyclin mRNAs (Fig. 3, lanes *10–13*). However, the oocytes that lacked cyclin mRNAs failed to show the series of "surface concentration waves" that normally follow parthenogenetic activation, and which are believed to correspond to activation and inactivation of MPF in activated eggs (Hara et al., 1980). This observation suggests that the inability to synthesize cyclin blocks the activation of MPF in the mitotic cell cycle in vivo as it does in vitro (Minshull et al., 1989).

Cyclin B Is Not Completely Degraded between the Two Meioses in Xenopus

Cyclin synthesis and degradation are required for the completion of each mitotic cell cycle (Minshull et al., 1989; Murray and Kirschner, 1989; Murray et al., 1989). The antisense ablation experiments described above show that *Xenopus* oocytes are able to pass through first and into second meiosis without new cyclin synthesis. Therefore, if cyclins are necessary for the induction of meiosis II, some of the oocyte's store of cyclin must survive the passage through meiosis I. We used immunoblotting to follow the fate of the B-type cyclins in oocytes in which protein synthesis had been inhibited by addition of cycloheximide just before GVBD. Fig. 4 shows that in this experiment, 70–80% of cyclin B2 was destroyed soon after GVBD, at the same time that H1 kinase levels fell (Fig. 4, *a* and *b*, compare lanes *6* and *7*). Cyclin B1 showed a similar decline (data not shown, but see Figs. *4* and *6* of the accompanying paper). The residual cyclin persisted for more than an hour after H1 kinase levels had fallen. Oocytes normally enter second meiosis about an hour after first meiosis (Gerhart et al., 1984). At this time, even in cycloheximide-treated oocytes, 20–30% of the B-type cyclin remained.

Within 2 h of GVBD the levels of residual cyclin began to fall and the oocytes showed dramatic changes in morphology (the "white puffball" phenotype) that we believe represent death and necrosis. We wanted to know whether this secondary loss of cyclin reflected the inability of oocytes to synthesize a MPF-stabilizing protein, such as c-*mos* (Daar et al., 1991) in the presence of cycloheximide. To answer this question, we investigated the level of cyclin proteins in oocytes that had progressed to second meiosis after ablation of their cyclin mRNAs, but with the synthesis of other proteins unaffected. Just like the cycloheximide-treated oocytes sampled

1 h after GVBD, these oocytes contained 20–30% of control levels of cyclin B2. Unlike the cycloheximide-treated oocytes, however, this lower level of B-type cyclin was still present as much as 16 h after progesterone addition (Fig. 4 *b*, lanes *13* and *14*), suggesting that after the initial destruction at the end of meiosis I, the B-type cyclins are stable as long as the synthesis of other proteins is allowed to continue. We propose that it is the cyclin that survives meiosis I that permits the second round of activation MPF, which in turn induces entry into meiosis II even when cyclin mRNAs have been ablated.

Discussion

The results in this paper show that *Xenopus* oocytes can mature in response to progesterone and pass through two meiotic divisions without new synthesis of cyclins A, B1, and B2. The simplest interpretation of this finding is that the two meiotic divisions are induced by mobilizing a preexisting store of maternal cyclin. This view is supported by the companion paper, which shows that stage VI oocytes contain a store of maternal cyclin B2 and B1 (Kobayashi et al., 1991). Our observations on meiosis I in *Xenopus* are reminiscent of those on clam and starfish oocytes, which contain a store of cyclin B and can enter meiosis I without protein synthesis (Labbé et al., 1989; Meijer and Guerrier, 1984; Westerndorf et al., 1989). Just as in *Xenopus*, however, the correct completion of meiosis II requires protein synthesis in these marine invertebrates (Houk and Epel, 1974; Picard et al., 1985).

The protein synthesis requirement for meiosis I in frogs and for meiosis II in frogs, clams, and starfish has been interpreted as a requirement for new cyclin synthesis (Pines and Hunt, 1987; Westendorf et al., 1989). This interpretation was extrapolated from the demonstration that mitotic cell cycles require new cyclin synthesis for each mitosis (Minshull et al., 1989; Murray and Kirschner, 1989), and that passage out of one M-phase into another requires the degradation of cyclin (Murray et al., 1989). However, our data clearly show that, at least in frogs, neither the protein synthesis requirement for meiosis I or II can be ascribed to a need to make new cyclin. We propose that in clams, as in frogs, the protein synthesis requirement for the correct completion of meiosis II may not represent a requirement for new cyclin, since clams also destroy only a part of their maternal cyclin B at first meiosis (Westendorf et al., 1989; J. Ruderman and E. Shibuya, personal communication).

What might then be the new protein or proteins that have to be synthesized in order to complete meiosis? As discussed in the introduction, there is persuasive evidence that c-*mos* synthesis is required for the induction of meiosis I and meiosis II (Sagata et al., 1988; Freeman et al., 1990), but none of the experiments so far rule out the possibility that other proteins besides c-*mos* are required for the induction of meiosis and, in particular, the successful completion of meiosis II. To demonstrate that c-*mos* synthesis alone was sufficient to induce maturation, it would be necessary to show that c-*mos* protein (as opposed to c-*mos* mRNA) can cause oocyte maturation in the presence of cycloheximide.

In early embryonic cell cycles, at least 90% of the cyclin is degraded at the exit from mitosis (Minshull et al., 1990; Westendorf et al., 1989). Furthermore, forms of cyclin that cannot be degraded block the inactivation of MPF at the end of meiosis II and also arrest embryonic mitosis at metaphase, showing that cyclin degradation is required for the inactivation of MPF in these divisions (Murray et al., 1989). The ability to exit from meiosis I in the absence of complete cyclin degradation thus suggests that a novel mechanism for inactivating MPF that does not involve cyclin degradation may exist at meiosis I. From the results in the accompanying paper, it would appear that cyclin B1 and cyclin B2 show different behavior at this stage of oocyte maturation. Perhaps the partial destruction of cyclin B2 is connected with the meiosis I to meiosis II transition, whereas the persistence of cyclin B1 may have little or no effect on this transition.

Our results show that cyclin A is not required for meiosis in frog oocytes, because unlike the B-type cyclins, there is no detectable store of cyclin A protein in oocytes (Kobayashi et al., 1991). Many events of the mitotic cell cycle occur normally in extracts where no A type cyclins are being made (Murray and Kirschner, 1989), prompting us to question the role of cyclin A in the early frog cell cycles. Since cyclin A in mammalian cells reaches a maximum abundance in S phase (Pines and Hunter, 1990; Giordano et al., 1989), it is tempting to speculate that the role of cyclin A may be to activate the replication machinery at the time of fertilization, after it has lain dormant throughout oogensis. A clear requirement for cyclin A synthesis has been demonstrated in the postblastoderm cell cycles of fly embryos (Lehner and O'Farrell, 1990). Since these cells arrest in G2, it suggests that cyclin A is required in these cell cycles for the induction of mitosis. We are currently trying to determine the roles of cyclin A and B in more detail.

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