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Progression from meiosis I to meiosis II in *Xenopus* oocytes requires *de novo* translation of the *mos*^{xe} protooncogene

(cell cycle/protein kinase/maturation-promoting factor/germinal vesicle breakdown)

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ABSTRACT **The meiotic maturation of *Xenopus* oocytes exhibits an early requirement for expression of the *mos*^{xe} protooncogene. The mos^{xe} protein has also been shown to be a component of cytostatic factor (CSF), which is responsible for arrest at metaphase of meiosis II. In this study, we have assayed the appearance of CSF activity in oocytes induced to mature either by progesterone treatment or by overexpression of *mos*^{xe}. Progesterone-stimulated oocytes did not exhibit CSF activity until 30–60 min after germinal vesicle breakdown (GVBD). Both the appearance of CSF activity and the progression from meiosis I to meiosis II were inhibited by microinjection of *mos*^{xe} antisense oligonucleotides just prior to GVBD. These results demonstrate a translational requirement for *mos*^{xe}, which is temporally distinct from the requirement for *mos*^{xe} expression at the onset of meiotic maturation. In contrast to progesterone-treated oocytes, oocytes that were induced to mature by overexpression of *mos*^{xe} exhibited CSF activity at least 3 hr prior to GVBD. Despite the early appearance of CSF, these oocytes were not arrested at meiosis I. These results indicate that, although CSF activity is capable of stabilizing maturation-promoting factor (MPF) at meiosis II and in cleaving embryos, it is incapable of stabilizing MPF prior to or at meiosis I. These studies show that the complex regulation of the cell cycle during meiosis differs significantly from the regulation of the cell cycle during mitosis.**

The stage VI oocyte of the frog *Xenopus laevis* is arrested in prophase of meiosis I. Exposure to progesterone induces meiotic maturation by allowing the oocyte to complete meiosis I and proceed to metaphase of meiosis II, at which point it remains arrested until fertilization. The activation of M phase or maturation-promoting factor (MPF) is required for oocyte maturation (1, 2). MPF activity is also necessary for mitosis (3, 4), and its existence has been demonstrated in a wide variety of eukaryotic organisms (for review, see ref. 5). MPF purified from *Xenopus* eggs contains the p34^{cdc2} serine/threonine protein kinase (6, 7), which is the catalytic subunit of histone H1 kinase. H1 kinase activity cycles during cell division in parallel with MPF activation and thus provides a biochemical assay of MPF activity (8, 9). Activation of p34^{cdc2} kinase leads to the phosphorylation of many M-phase substrates that are presumably involved in cell division (for a review, see ref. 10).

The periodicity of MPF activation may be regulated by cyclin proteins (11, 12), which were originally characterized as proteins synthesized during interphase and specifically degraded at each mitosis (13). Cyclin synthesis is required for the activation of MPF in *Xenopus* egg extracts (14, 15). Furthermore, if cyclin degradation is prevented, MPF activity remains high, and the cell cycle arrests in metaphase (16). Thus, the factors regulating MPF activity are responsible for controlling entry into and exit from M phase (for reviews, see refs. 17–19).

In *Xenopus*, protein synthesis is required for the initiation of meiosis I and also meiosis II (4, 20), even though stage VI oocytes already contain both p34^{cdc2} and cyclin (12, 21). These proteins are partially complexed in an inactive form of MPF (preMPF) that appears to be normally inhibited by a protein phosphatase activity called "INH" (22, 23). These observations indicate a translational requirement, both for the initiation of maturation and for progression to meiosis II, for a regulatory factor(s) other than cyclin. One candidate regulator of MPF activity is the serine/threonine protein kinase encoded by the *mos*^{xe} protooncogene. Translation of *mos*^{xe} is required for meiotic maturation induced by progesterone (24). Furthermore, microinjection of mos^{xe} RNA into stage VI oocytes induces germinal vesicle breakdown (GVBD) in the absence of progesterone stimulation (25, 26).

Cytostatic factor (CSF) activity was originally demonstrated by the transfer of cytoplasm from unfertilized amphibian eggs into the blastomeres of cleaving two-cell embryos, resulting in a stable metaphase arrest in the injected blastomeres (1). CSF activity is thought to be responsible for the stabilization of MPF at metaphase of meiosis II and has been shown to disappear upon fertilization of the egg (27, 28). Recent studies indicate that the mos^{xe} protein shares many of the properties of CSF. Progesterone treatment induces the expression of endogenous mos^{xe} protein which, upon fertilization, is rapidly degraded (29). The microinjection of mos^{xe} RNA or viral v-mos RNA into a dividing embryo produces a stable metaphase arrest (30, 31). In addition, antibodies directed against the mos^{xe} protein immunodeplete CSF activity from *Xenopus* egg extracts (30). These studies indicate that the mos^{xe} protein is an essential component of CSF.

In this study, we have assayed the appearance of CSF activity and H1 kinase activity in oocytes induced to mature either by progesterone treatment or by microinjection of mos^{xe} RNA. These studies show that the synthesis of mos^{xe} protein is a translational prerequisite for meiosis II, as it is for meiosis I. These studies also reveal that CSF activity is incapable of stabilizing MPF prior to or at meiosis I, in contrast to its ability to stabilize MPF at meiosis II and in cleaving embryos. These results exemplify some of the significant differences in regulation of the cell cycle during meiosis versus mitosis.

MATERIALS AND METHODS

Oocyte Microinjections and GVBD Assays. Stage VI oocytes, isolated from *Xenopus* females primed with pregnant mare serum gonadotropin, were induced to mature by microinjection of either 50 nl of *in vitro* transcribed mos^{xe}

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Abbreviations: GVBD, germinal vesicle breakdown; MPF, maturation-promoting factor; CSF, cytostatic factor.

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RNA (1.0 mg/ml) (25) or 25 nl of cyclin B protein (3 mg of total protein per ml), or by incubation with progesterone (10 μg/ml). The cyclin protein was sea urchin cyclin B (16), which was prepared as a recombinant fusion protein overexpressed in *Escherichia coli* (provided by C. Smythe). GVBD was monitored by the appearance of a white spot in the animal hemisphere. Oocytes that had undergone GVBD within 15 min of each other were assayed together for subsequent time points.

Assay of CSF Activity. Fertilized *Xenopus* eggs were prepared as described (31). At the time first cleavage of the embryo was observed, cytoplasm from donor oocytes was withdrawn, and 25 nl was injected into one of the blastomeres as described (1). CSF arrest was characterized by a stable cleavage arrest in the injected blastomere, which showed no signs of pseudo-cleavage or deterioration over the next 6 hr.

Assay of Histone H1 Kinase Activity. Oocytes that had undergone GVBD within 15 min of each other were assayed together for time points after GVBD. Oocytes were lysed in buffer containing 80 mM β-glycerophosphate (pH 7.8), 100 mM NaCl, 15 mM $MgCl_2$, 10 mM EGTA, 50 μM ATP, and 10 μg of leupeptin and of aprotinin per ml (1 oocyte per 12.5 μl). Aliquots (5 μl) of the clarified lysates were mixed 1:1 with buffer containing 40 mM Hepes (pH 7.3), 20 mM $MgCl_2$, 10 mM EGTA, 0.2 mg of histone H1 per ml, 10 μM cAMP-dependent protein kinase inhibitor, and 5 μCi (185 kBq) of [γ-^{32}P]ATP. After 10 min at room temperature, the reactions were stopped, and radiolabeled histones were resolved by SDS/PAGE and detected by autoradiography. Each gel lane represents the H1 kinase activity from 0.5 oocyte. In some experiments protein synthesis was blocked by incubating the oocytes with cycloheximide at 100 μg/ml (20). The specific inhibition of mos^{xe} protein expression was performed by injecting 50 nl of *mos*^{xe} antisense oligonucleotide (2.0 mg/ml) into each oocyte as described (31).

RESULTS

Fig. 1*A* shows the oscillation in MPF activity, as reflected by H1 kinase activity, between meiosis I and meiosis II. Oocytes were treated with progesterone and allowed to undergo GVBD. Once GVBD was observed, groups of oocytes were collected every 15 min to synchronize samples for assaying H1 kinase activity at given time points after GVBD. H1 kinase activity was high at GVBD and then decreased between 30 and 60 min after GVBD. After 60 min, H1 kinase activity increased again and remained elevated. H1 kinase activity was also assayed in progesterone-treated oocytes that were incubated with cycloheximide (100 μg/ml) 30 min prior to GVBD (Fig. 1*B*). The H1 kinase activity was observed to drop after GVBD but did not subsequently increase. These results are consistent with earlier reports that characterized the oscillations of MPF activity and the requirement for translation between meiosis I and meiosis II (4).

Progesterone-Stimulated Oocytes Exhibit CSF Activity Only After GVBD. The presence of CSF activity has been demonstrated after GVBD in unfertilized eggs (1, 28). Recent reports have indicated that the mos^{xe} protein kinase demonstrates many of the characteristics of CSF (29, 30). Although the level of mos^{xe} protein increases early in maturation (26, 29), the presence of CSF activity prior to GVBD has not been reported. Therefore, we assayed oocytes for CSF activity throughout maturation. Cytoplasm was withdrawn from oocytes at different stages of maturation and injected into cleaving embryos to assay CSF arrest. CSF activity was not observed prior to GVBD and was first detected between 30 and 60 min after GVBD (Fig. 2). This result indicates that, despite the early appearance of endogenous mos^{xe} protein during maturation, CSF activity does not appear until after GVBD.

Appearance of CSF Activity Requires a Late Phase of Translation of *mos*^{xe}. To test whether the expression of additional mos^{xe} protein was necessary for meiosis II and the appearance of CSF activity, antisense oligonucleotides specific for the mos^{xe} transcript were injected into progesterone-treated oocytes 30 min prior to GVBD. The injection of *mos*^{xe} antisense oligonucleotides has previously been demonstrated to block the *de novo* synthesis of the mos^{xe} protein (24, 31). The H1 kinase activity in control oocytes decreased 30–60 min after GVBD and began to increase again 60 min after

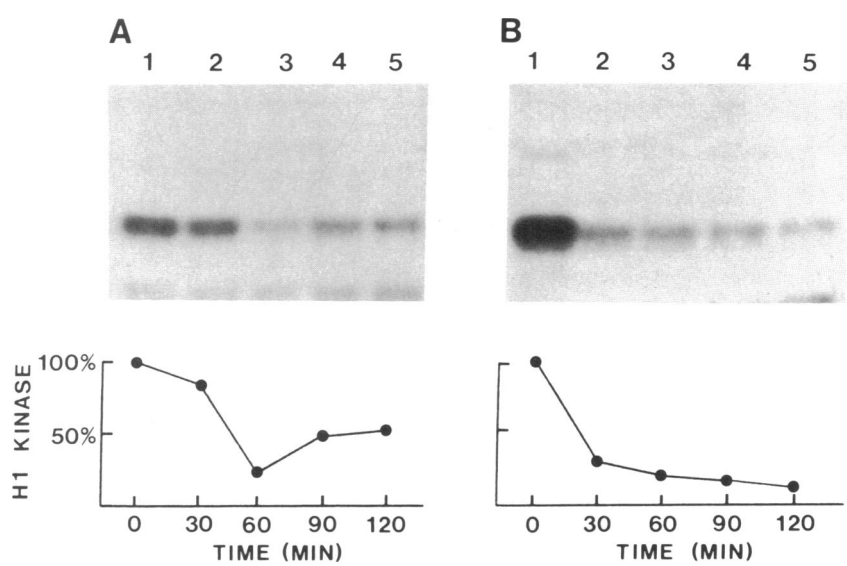

FIG. 1. (*A* and *B Upper*) H1 kinase activity of progesterone-treated oocytes in the absence (*A*) or presence (*B*) of cycloheximide. (*A*) Oocytes were induced to mature by progesterone treatment and assayed for H1 kinase activity at the following times: at GVBD (lane 1), 30 min after GVBD (lane 2), 60 min after GVBD (lane 3), 90 min after GVBD (lane 4), and 120 min after GVBD (lane 5). (*B*) H1 kinase activity was assayed in progesterone-treated oocytes as in *A* except that 30 min prior to GVBD, these oocytes were incubated in cycloheximide at 100 μg/ml. The lanes correspond to time points as described for *A*. The predominant band in each lane represents phosphorylated histone H1 resolved by SDS/PAGE and detected by autoradiography. (*Lower*) Bands of phosphorylated histone H1 for the gel shown were quantitated by laser densitometry and are presented graphically. The time is shown with respect to GVBD. The H1 kinase activity is normalized to that observed at GVBD (100%) in lane 1.

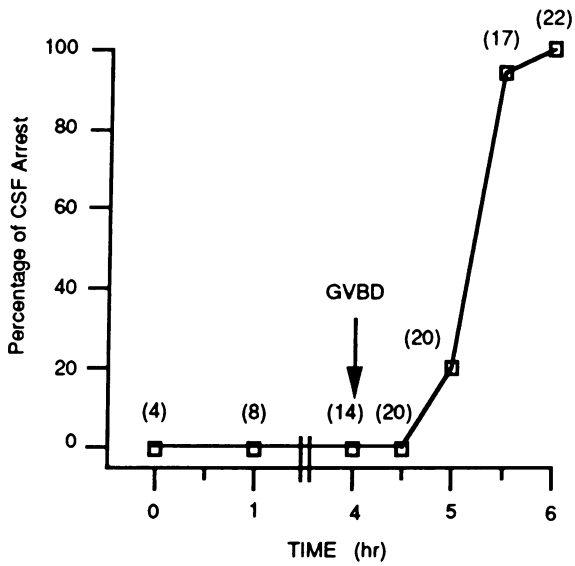

FIG. 2. Appearance of CSF activity in progesterone-treated oocytes. Oocytes were induced to mature by progesterone treatment at 0 hr and then assayed for CSF activity at various times during maturation. At the indicated times, cytoplasm was withdrawn from the maturing oocytes and microinjected into one blastomere of cleaving two-cell embryos. Oocytes that had undergone GVBD within 15 min of each other were assayed together for time points after GVBD. Cleavage arrest of the recipient blastomeres indicated the presence of CSF activity in the donor oocytes. Numbers in parentheses indicate the total number of blastomeres microinjected at each time point. GVBD occurred at 4 hr as indicated.

GVBD (Fig. 3*A*). The oocytes injected with *mos*^{xe} antisense oligonucleotides also exhibited a decrease in H1 kinase activity 30 min after GVBD, which decline continued through 120 min post-GVBD (Fig. 3*B*). These oocytes were also assayed for CSF activity between 70 and 90 min after GVBD. While cytoplasm from control oocytes exhibited CSF activity by this time, the oocytes injected with antisense oligonucleotides did not demonstrate any CSF activity (Table 1). The microinjection of *mos*^{xe} antisense oligonucleotides also inhibited the appearance of CSF activity in oocytes that were induced to mature by the microinjection of sea urchin cyclin B protein in place of progesterone treatment (Table 1). These results show that, in the absence of *de novo* expression of *mos*^{xe}, oocytes undergo GVBD but neither progress to meiosis II nor exhibit CSF activity.

Oocytes Matured by Overexpression of *mos*^{xe} Are Not Arrested at Meiosis I. Overexpression of mos^{xe} protein initiates meiotic maturation, possibly through the stabilization of MPF (25, 26). The ability of mos^{xe} to stabilize MPF may also be responsible for its CSF activity (30). Therefore, we examined whether overexpression of *mos*^{xe} in maturing oocytes might lead to arrest at meiosis I. Oocytes were induced to mature either by progesterone treatment or by microinjection of mos^{xe} RNA. Oocytes were then assayed for H1 kinase activity at time points throughout maturation. The H1 kinase activity in the progesterone-treated oocytes increased rapidly at GVBD and then decreased to a minimum level 60 min after GVBD before increasing once again as the oocytes reached meiosis II (Fig. 4*A*). The H1 kinase activity profile of oocytes matured by the overexpression of mos^{xe} protein was almost identical to those matured by progesterone (Fig. 4*B*). This indicates that overexpression of *mos*^{xe} was incapable of stabilizing MPF and arresting maturation prematurely at meiosis I.

Oocytes Matured by Overexpression of *mos*^{xe} Exhibit CSF Activity Throughout Maturation. The oocytes described in the above experiment were also examined for the appearance of CSF activity. Unlike progesterone-treated oocytes, oocytes matured by the microinjection of mos^{xe} RNA exhibited CSF activity prior to GVBD and throughout meiosis,

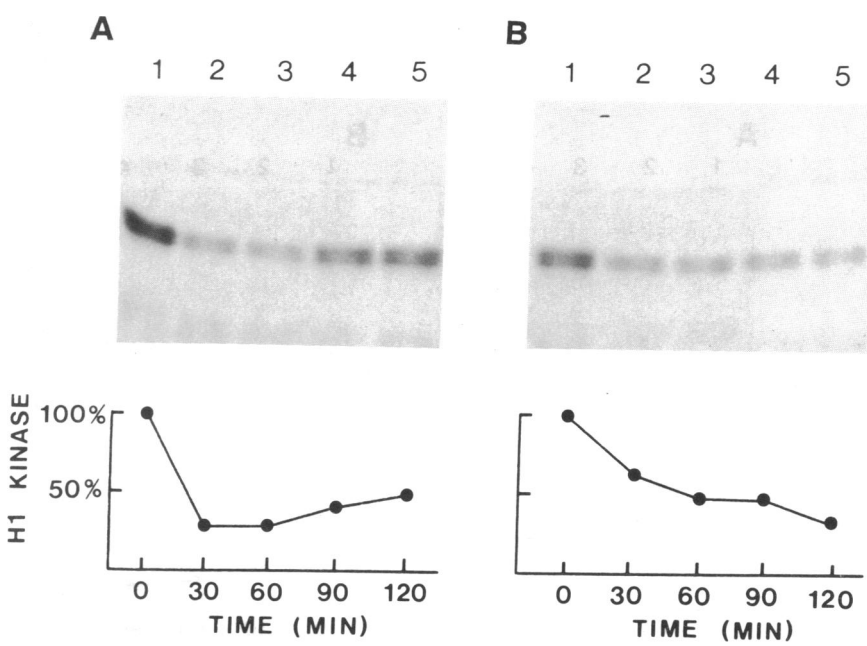

FIG. 3. (*A* and *B Upper*) H1 kinase activity of progesterone-treated oocytes in the absence (*A*) or presence (*B*) of microinjected *mos*^{xe} antisense oligonucleotide. (*A*) Control oocytes were induced to mature by progesterone treatment and assayed for H1 kinase activity. Oocytes were assayed for H1 kinase activity at the following times: at GVBD (lane 1), 30 min after GVBD (lane 2), 60 min after GVBD (lane 3), 90 min after GVBD (lane 4), and 120 min after GVBD (lane 5). (*B*) H1 kinase activity was assayed in progesterone-treated oocytes as in *A* except that 30 min prior to GVBD, each of these oocytes was microinjected with 50 nl of *mos*^{xe} antisense oligonucleotides (2 mg/ml). The lanes correspond to time points as described for *A*. Quantitation of H1 kinase activity is as described for Fig. 1. (*Lower*) When the results from independent experiments were compared, the following average values were obtained: for progesterone-stimulated oocytes, 33% H1 kinase activity was observed at 60 min, which rose to 55% H1 kinase activity at 120 min (*A*); for oocytes injected with *mos*^{xe} antisense oligonucleotides, 36% H1 kinase activity was observed at 60 min which declined to 26% H1 kinase activity at 120 min (*B*).

Table 1. Effect of *mos*^{xe} antisense oligonucleotides on CSF activity

	% of CSF arrest	
	Noninjected	Injected
Progesterone	100% (16)	0% (19)
Cyclin protein	93% (43)	16% (38)

Oocytes were matured by progesterone treatment or by microinjection of sea urchin cyclin B protein. Thirty minutes prior to GVBD, half of the oocytes were injected with *mos*^{xe} antisense oligonucleotide. Seventy to 90 min after GVBD, the oocytes were assayed for CSF activity. The numbers in parentheses indicate the number of blastomeres injected for each condition.

beginning as early as 60 min after injection and continuing throughout the post-GVBD drop in H1 kinase activity (Fig. 5). Thus, although expression of *mos*^{xe} is required for the stabilization of MPF at meiosis II and for the appearance of CSF activity, it does not appear to stabilize MPF in the oocyte even when CSF activity is present during meiosis I.

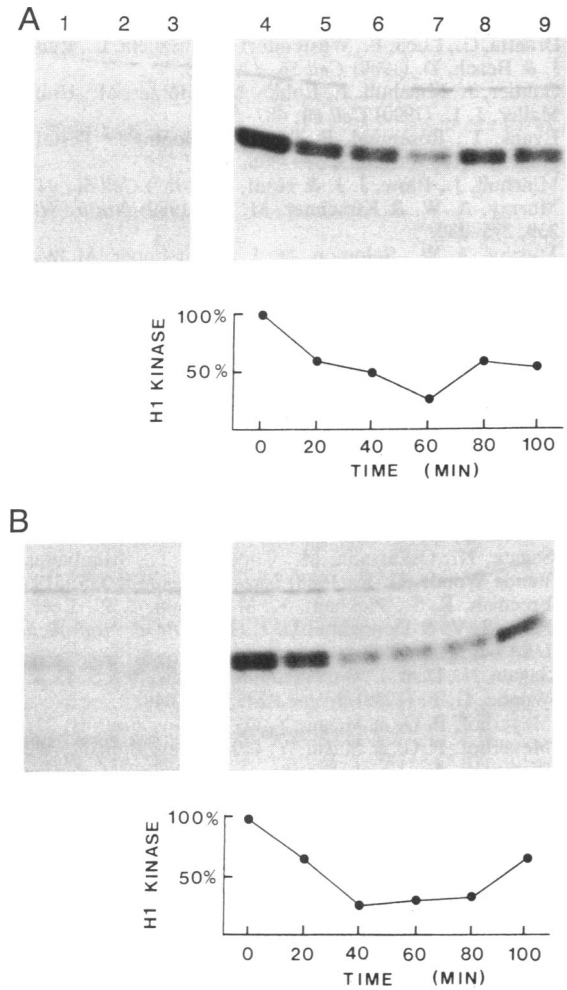

FIG. 4. H1 kinase activity of oocytes matured either by progesterone treatment (*A*) or by microinjection of mos^{xe} RNA (*B*). (*A*) Oocytes were induced to mature by progesterone treatment and assayed for H1 kinase activity at the following times: 1 hr after progesterone treatment (lane 1), 2 hr after progesterone treatment (lane 2), 3 hr after progesterone treatment (lane 3), at GVBD (lane 4), 20 min after GVBD (lane 5), 40 min after GVBD (lane 6), 60 min after GVBD (lane 7), 80 min after GVBD (lane 8), and 100 min after GVBD (lane 9). (*B*) H1 kinase activity was assayed in oocytes induced to mature by microinjection with mos^{xe} RNA. The lanes correspond to time points as described for *A*. Quantitation of H1 kinase activity at GVBD and later times is as described for Fig. 1.

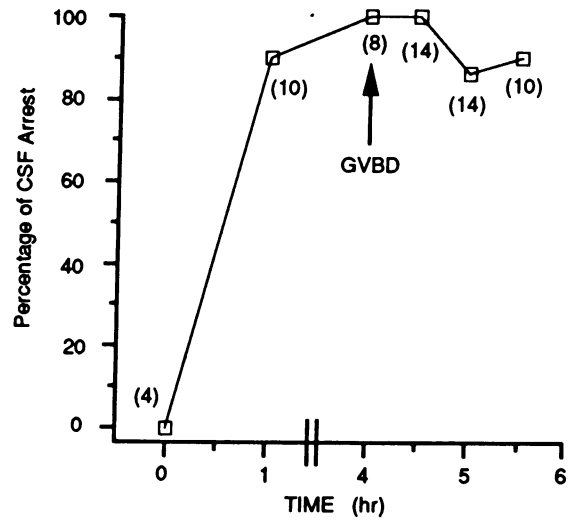

FIG. 5. Appearance of CSF activity in oocytes induced to mature by microinjection of mos^{xe} RNA. Oocytes were microinjected with mos^{xe} RNA at 0 hr and then assayed for CSF activity at various times during maturation as described for Fig. 2. Cleavage arrest of the recipient blastomeres indicated the presence of CSF activity in the donor oocytes. Numbers in parentheses indicate the total number of blastomeres microinjected at each time point. GVBD occurred at 4 hr as indicated.

DISCUSSION

Histone H1 kinase activity has been used as an indirect measurement of MPF activity (8, 9, 32), and the fluctuations in H1 kinase activity between meiosis I and meiosis II correlate well with the reported fluctuations in MPF activity during this period. Furthermore, cycloheximide inhibits the activation of H1 kinase activity after the completion of meiosis I, consistent with its inhibition of MPF activation (4). Together, these studies show that H1 kinase activity can be used to indicate the progression of the cell cycle from meiosis I to meiosis II.

Previous work has indicated that the synthesis of the mos^{xe} protein is necessary for the initiation of meiosis (24), and in addition, mos^{xe} protein constitutes an essential component of CSF (29, 30). The level of endogenous mos^{xe} protein has been shown to increase early in maturation and may persist through meiosis II (26, 29). However, in this study, we did not detect CSF activity in progesterone-treated oocytes until significantly after GVBD. Furthermore, H1 kinase levels were found to remain low until just prior to GVBD, whether induced by progesterone or by the microinjection of mos^{xe} RNA. These results indicate that, despite the early presence of endogenous mos^{xe} protein in maturing oocytes and despite the overexpression of *mos*^{xe} in microinjected oocytes, significant stabilization or activation of endogenous MPF in the oocyte does not occur until close to GVBD. This suggests the existence of additional factors involved in the regulation of MPF activation.

After the completion of meiosis I, protein synthesis is necessary for the activation of MPF and subsequent metaphase arrest at meiosis II (4). When progesterone-stimulated oocytes were microinjected with mos^{xe} antisense oligonucleotides just prior to GVBD, the reactivation of MPF leading to meiosis II was inhibited; similarly, the appearance of CSF activity was also inhibited. Furthermore, when oocytes were microinjected with sea urchin cyclin B protein in the presence of *mos*^{xe} antisense oligonucleotides, GVBD was induced but the subsequent appearance of CSF activity was inhibited. These results show a translational requirement for *mos*^{xe} that is temporally distinct from the previously described require-

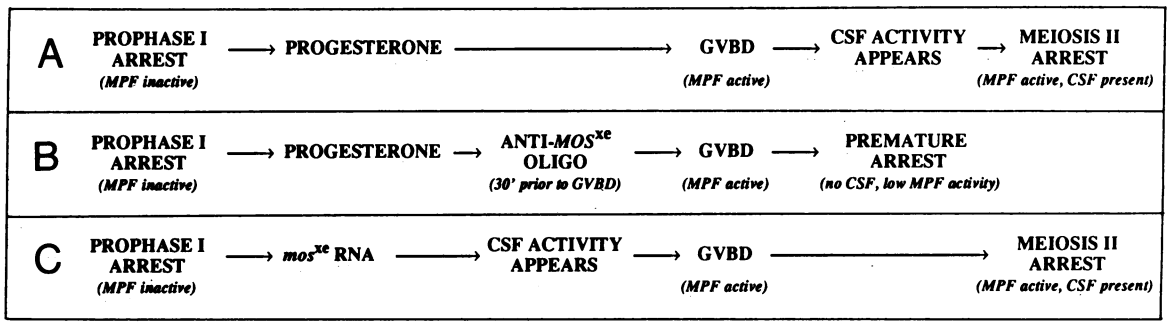

FIG. 6. Effects of abnormal mos^{xe} expression on oocyte maturation. (*A*) Normal progression of oocyte maturation in response to progesterone. (*B*) Effects on the appearance of CSF activity and MPF activation when antisense mos^{xe} oligonucleotides are injected just prior to GVBD. (*C*) Summary of the results when meiotic maturation is initiated by overexpression of mos^{xe}.

ment for mos^{xe} translation at the onset of meiotic maturation (24).

These results, outlined schematically in Fig. 6, are consistent with reports concerning mos^{xe} protein function during mouse oocyte maturation. During mouse oocyte maturation, *de novo* protein synthesis is only required after GVBD (33). The microinjection of mos^{xe} antisense oligonucleotides into mouse oocytes does not inhibit GVBD but does block progression to meiosis II (34). This is consistent with our results in *Xenopus* where *de novo* synthesis of the mos^{xe} protein is required for meiosis II.

Oocytes that are induced to mature by overproduction of mos^{xe} protein exhibit CSF activity as early as 60 min after RNA injection, and this CSF activity persists through both meiosis I and II. However, despite the early appearance of CSF activity during overexpression of mos^{xe}, MPF levels in the oocyte are not prematurely activated, nor do these oocytes undergo CSF arrest at meiosis I. Thus, although CSF activity is capable of stabilizing MPF at meiosis II and in cleaving embryos, it is incapable of stabilizing endogenous MPF prior to or at meiosis I. This suggests that an unidentified activator or substrate for CSF may be absent in the maturing oocyte prior to meiosis I, although present in unfertilized eggs and M-phase blastomeres. In conclusion, these studies indicate that the complex regulation of the cell cycle during meiosis may differ significantly from the regulation of the cell cycle during mitosis.

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