A Characterization of Cytostatic Factor Activity from *Xenopus* Eggs and c-*mos*-transformed Cells

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Abstract. In *Xenopus* oocytes, the *mos* proto-oncogene product is required during meiosis I for the activation of maturation promoting factor (MPF) and the subsequent breakdown of the germinal vesicle (GVBD). In addition, the *mos* product has been shown to be a candidate "initiator" of meiotic maturation and is an active component of cytostatic factor (CSF), an activity responsible for metaphase II arrest. Here we demonstrate that pp39^{mos} is required throughout oocyte maturation. We found that in progesterone stimulated oocytes, depletion of *mos* RNA immediately before GVBD terminally decreased MPF. Likewise, oocytes depleted of *mos* RNA and induced to mature with crude MPF proceeded through GVBD, but lacked the MPF activity required to arrest mature oocytes at metaphase II. Thus, during maturation the *mos* product is required, directly or indirectly, to sustain MPF activity. On the other hand, mouse NIH/3T3 cells transformed by the constitutive expression of pp39^{mosxe} possessed CSF activity but lacked constitutive levels of MPF or its associated histone H1 kinase activity. Moreover, cytosols prepared from transformed NIH/3T3 cells or *Xenopus* eggs had similar levels of CSF activity, but pp39^{mos} levels were >40-fold higher in the transformed cell extract. These analyses show that maintenance of CSF during interphase does not result in the maintenance of MPF.

FULLY grown *Xenopus* oocytes arrested in prophase of meiosis I (Masui and Clarke, 1979) are induced to mature with progesterone, resulting in the activation of maturation promoting factor (MPF)[1] and the progression through meiosis (Masui and Markert, 1971; Ford, 1985). MPF is a cytoplasmic protein kinase activity that leads to nuclear envelope breakdown as well as chromosome condensation (Miake-Lye et al., 1983; Maller, 1985) and consists of two proteins, the homologue of the p34^{cdc2} protein from *Schizosaccharomyces pombe* (Gautier et al., 1988; Dunphy et al., 1988) and a cyclin protein (Draetta et al., 1989; Meijer et al., 1989; Pines and Hunter, 1989; Gautier et al., 1990). Progesterone treatment of *Xenopus* oocytes rapidly induces the synthesis of the *mos* proto-oncogene product, pp39^{mos}, that precedes MPF activation and germinal vesicle breakdown (GVBD) (Sagata et al., 1989*a*). Moreover, the introduction of c-*mos*^{xe} RNA into fully grown oocytes triggers the activation of MPF and GVBD (Sagata et al., 1989*a*; Freeman et al., 1989).

In *Xenopus* oocytes, pp39^{mos} is required for MPF activation and GVBD induced by progesterone (Sagata et al., 1988; Barrett et al., 1990), while in mouse oocytes, pp39^{mos} is required only after GVBD (O'Keefe et al., 1989; Paules et al., 1989). Moreover, the *mos* product is expressed throughout maturation in both *Xenopus* and mouse oocytes undergoing maturation (Sagata et al., 1988; Watanabe et al., 1989; Paules et al., 1989). The above evidence suggests that pp39^{mos} function is required throughout meiotic maturation (Paules et al., 1989). Recently, pp39^{mos} has been identified as an active component of cytostatic factor (CSF) (Sagata et al., 1989*b*), a calcium-sensitive activity believed to be responsible for the arrest of an unfertilized egg at metaphase II and for the stabilization of MPF (Masui and Clarke, 1979; Newport and Kirschner, 1984; Sagata et al., 1989*b*; Murray et al., 1989). Here we provide evidence that the *mos* product is required throughout meiotic maturation for the maintenance of MPF. In contrast, we show that CSF activity is present in c-*mos*^{xe}-transformed mouse NIH/3T3 cells during interphase, while MPF activity is not.

Materials and Methods

Frogs, Oocytes, Eggs, and Embryos

Xenopus laevis females were purchased from Xenopus I (Ann Arbor, MI). Oocytes were surgically removed and defolliculated by incubation in modified Barth solution (MBS; 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 10 mM Hepes [pH 7.5], 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$) containing collagenase A (Boehringer Mannheim Biochemicals, Indianapolis, IN) (2 mg/ml) for 2 h (Gurdon and Wickens, 1983). The oocytes were washed in MBS several times before the stage VI

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1. *Abbreviations used in this paper*: CSF, cytostatic factor; GVBD, germinal vesicle breakdown; MPF, maturation promoting factor.

(Dumont, 1972) oocytes were isolated. To obtain eggs, frogs were injected with 100 IU of pregnant mare serum gonadotropin 72 h before an injection of 625 IU of human chorionic gonadropin into the dorsal lymph sac. Frogs were maintained 12–14 h in 1× MMR (0.1 M NaCl, 2 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 0.1 mM EDTA, 5 mM Hepes [pH 7.8]) at 18°C, and the laid eggs were transferred to a 100-mM dish containing 1× MMR. To obtain embryos, eggs were placed in 100% Ringers' solution (0.1 M NaCl, 1.8 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 4 mM $NaHCO_3$). Minced testes were added to the dish containing the eggs, and 2 vol of H_2O were added to dilute the Ringers' solution to 0.3×. After 15 min, eggs were dejellied in 0.3× MMR containing 2% cysteine (pH 7.9). The eggs were washed several times with 0.3× MMR and cultured in 0.3× MMR containing 5% Ficoll 400.

Oligodeoxyribonucleotide Injections

18 h after fully grown oocytes were isolated, microinjections were performed with 40 nl of a mixture of either antisense or sense *mos*-specific oligodeoxyribonucleotides (oligonucleotides; 3 mg/ml), denoted by A±, B±, C±, D± as described by Sagata et al. (1988). Subsequent injections of crude MPF were performed 4 h later.

MPF Assay

Crude MPF extracts were prepared by homogenizing groups of 10–20 oocytes or embryos in 20–40 μl of extraction buffer (80 mM sodium β-glycerophosphate, 20 mM EGTA, 15 mM $MgCl_2$, 20 mM Hepes [pH 7.2], 1 mM ATP, 1 mM DTT, 5 mM NaF). The homogenate was centrifuged at 16,000 *g* for 5 min at 4°C, and the supernatant was used for microinjections. Groups of 12–14 oocytes were incubated in 1× MBS containing cycloheximide (10 μg/ml) for 1 h and then injected with 40 nl of the supernatant from each appropriate donor group. The recipient oocytes were cultured 3–4 h in the presence of cycloheximide, then examined for GVBD. GVBD was scored by the observation of a white spot at the animal pole. The oocytes were fixed in 10% TCA for 10 min, then dissected and examined under a binocular microscope for the presence or absence of the germinal vesicle.

CSF Assay

Either eggs or NIH/3T3 cells expressing *tpr-met*, *ras*, or *Xenopus mos* were washed with CSF extraction buffer (0.25 M sucrose, 0.2 M NaCl, 5 mM $MgSO_4$, 2.5 mM EGTA, 20 mM Hepes [pH 6.5], 10 mM NaF, 80 mM sodium β-glycerophosphate, 2 mM DTT, 6 mM ATP). The CSF extraction buffer was removed, and the samples were crushed at 100,000 *g* at 2°C for 1.5 h. The supernatant was removed and 40 nl was injected into either one or both blastomeres of a 2-cell embryo. The embryos were maintained in 0.3× MMR containing 5% Ficoll 400 for 4–6 h and examined for cleavage.

Histone H1 Kinase Assay

2 μl of extract was transferred into 50 μl of extraction buffer (80 mM sodium β-glycerophosphate, 20 mM EGTA, 50 mM $MgCl_2$, 20 mM Hepes [pH 7.2], 1 mM DTT, 2.5 mM PMSF, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 10 μM protein kinase inhibitor). The histone H1 kinase reaction was performed by adding 10 μl of the sample to 6 μl of a mixture containing 1 μg/μl histone H1, 1 mM ATP, and 1 μCi [γ^{32}P]ATP. The reaction was incubated at room temperature for 15 min and then stopped by the addition of an equal volume of 2× sample buffer (4% SDS, 20% glycerol, 150 mM Tris-HCl [pH 6.8], 0.02% bromophenol blue, 5% β-mercaptoethanol). Samples were resolved by SDS-PAGE on a 12% gel. Gels were fixed in 40% methanol, 5% acetic acid, then dried, and exposed to film.

Immunoprecipitation and Western Blotting Analysis

From either 300 *Xenopus* eggs or 4 × 10^7 c-*mos*^{xe}-transformed NIH/3T3 cells or nontransformed NIH/3T3 cells, a CSF extract was prepared as described above. To 100 μl of each extract, an equal amount of 2× RIPA buffer plus protease inhibitors (2.0% NP-40, 1.0% sodium deoxycholate, 0.2% SDS in PBS plus 5.0 mM PMSF and 120 kallikrein inhibitor units of aprotinin per milliliter) was added. These samples were subjected to immunoprecipitation with the 5S monoclonal *Xenopus mos* antibody as described (Sagata et al., 1989*a*). Immune complexes were resolved by 3–17% gradient SDS-PAGE. Western transfer was performed as described (Burnette, 1981). pp39^{mos} was detected with 5S monoclonal antibody and alkaline phosphatase-conjugated goat anti-mouse IgG.

Immunoprecipitation and In Vitro Kinase Assay

To 100 μl of CSF extract, 5S monoclonal antibody (Sagata et al., 1989*a*) was added, incubated for 3 h, and complexed with 10% protein A-Sepharose in CSF buffer. The pellet was washed five times in wash buffer (1% NP-40, 150 mM NaCl, 1 mM EDTA, 10 mM sodium phosphate, 20 μM leupeptin, 2 mM PMSF, 10 μM pepstatin, 2 mM dithiothreitol, 120 kallikrein inhibitor units of aprotinin per milliliter). Then 50 μl of solution A (0.1% NP-40, 150 mM NaCl, 10 mM sodium phosphate [pH 7.2], 2 mM DTT, 1 mM sodium pyrophosphate) and 5 μl of 2 mM quercitin (Sigma Chemical Co., St. Louis, MO) was added to the immune complex and incubated on ice for 10 min. After the addition of 50 μl of solution B (0.1% NP-40, 150 mM NaCl, 10 mM sodium phosphate [pH 7.2], 2 mM DTT, 1 mM sodium pyrophosphate, 15 mM $MnCl_2$, 20 μM ATP, 50 μCi of [γ^{32}P]ATP) the reaction mix was incubated at room temperature for 10 min. 2× sample buffer was added, and the sample was resolved on a 12% SDS-polyacrylamide gel. Gels were fixed, dried, and exposed to film.

Cell Culture

NIH/3T3 cells transformed by the *Xenopus mos* product expressed from a plasmid containing the *Xenopus mos* gene (Sagata et al., 1988) just downstream of a Moloney sarcoma virus long terminal repeat (Yew et al., 1991) were cultured in DMEM containing 8% calf serum. NIH/3T3 cells transformed by either the activated H-*ras* oncogene (gift from Dr. Youyong Lu) or the *tpr-met* oncogene (Park et al., 1986) were also cultured in DMEM with 8% calf serum. Mitotic cell cycle arrest was achieved by the addition of medium containing 0.4 μg/ml of nocodozole (Calbiochem-Behring Corp., Palo Alto, CA) and incubating the cell culture 7.5–20 h at 37°C. Mitotic cells were collected by shaking off the loosely attached cells and centrifugation at 1,500 *g* for 5 min. The mitotic index was determined to be ~80% by light microscopy.

Results

Antisense Oligonucleotides Inhibit MPF Activation after GVBD

The induction of MPF and GVBD by progesterone or insulin is blocked by the injection of *mos*-specific antisense oligonucleotides (Sagata et al., 1988). To examine whether pp39^{mos} synthesis is required after GVBD, fully grown stage VI oocytes were microinjected with a mixture of either antisense or sense *mos*-specific oligonucleotides and subsequently injected with MPF to overcome the block to GVBD imposed by the depletion of pp39^{mos}. Oocytes were cultured 8 h following GVBD to allow for progression through meiosis I and arrest at metaphase II (Gerhart et al., 1984). Cytosolic ex-

Table I. Lack of MPF Activity after GVBD in Antisense Oligonucleotide Injected Oocytes

	Percent GVBD	
Treatment	Donor	Recipient
* Antisense oligonucleotide plus MPF	100	0
Sense oligonucleotide plus MPF	100	100
‡ Progesterone plus antisense oligonucleotide	100	0
Progesterone plus sense oligonucleotide	100	100

* 8 h after GVBD, cytosolic extracts were prepared from oocytes that had been injected with crude MPF extracts subsequent to either *mos* sense or antisense oligonucleotide injection as described in Materials and Methods. These donor extracts were injected into recipient cycloheximide-treated (10 μg/ml) oocytes and scored for GVBD as described in Materials and Methods.
‡ 8 h after GVBD, cytosolic extracts were prepared from oocytes that were treated with progesterone and subsequently injected with either sense or antisense *mos* oligonucleotides as described in Materials and Methods. These donor extracts were injected into recipient cycloheximide-treated (10 μg/ml) oocytes and scored for GVBD as described in Materials and Methods.

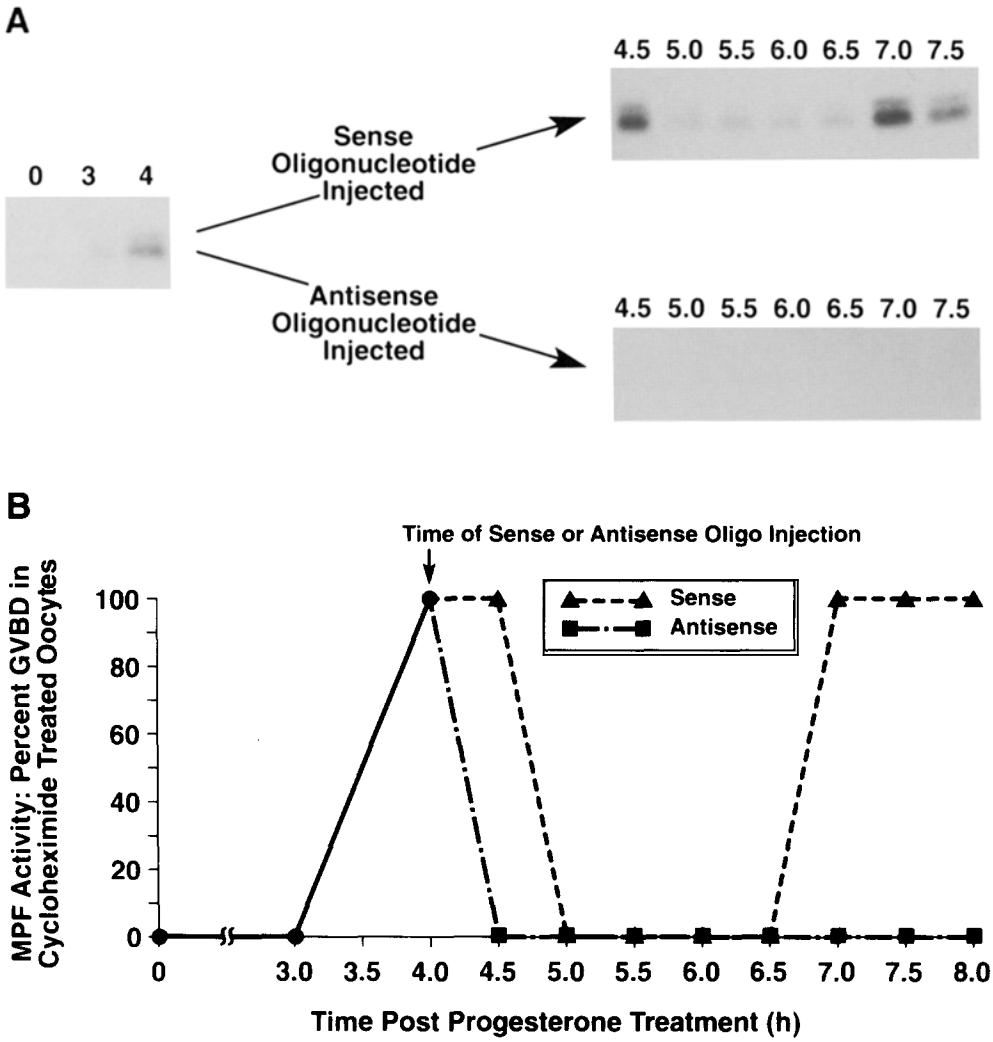

Figure 1. Kinetics of MPF and histone H1 kinase activity in the presence of sense or antisense oligonucleotides. Stage VI oocytes were treated with progesterone. 4 h later, oocytes were injected with 80 ng of either sense or antisense *mos* oligonucleotides. Cytosolic extracts were prepared from groups of six oocytes at the indicated times post-progesterone addition, then assayed for histone H1 kinase activity (*A*) or MPF activity (*B*).

tracts were then prepared and tested for MPF activity by microinjection into cycloheximide-treated fully grown oocytes (Table I). MPF can induce GVBD even in the presence of cycloheximide (Wasserman and Masui, 1975; Cyert and Kirschner, 1988). Our experiments showed that *mos* RNA depletion prevented the appearance of MPF 8 h after GVBD (Table I). Moreover, there was a 50% increase in the time required to reach GVBD in the *mos* RNA depleted oocytes (data not shown). These analyses demonstrated that *mos* was required after GVBD, even in the presence of injected MPF, and MPF-induced GVBD was accelerated in the presence of endogenous pp39^{mos}.

We tested whether pp39^{mos} synthesized before GVBD, was sufficient to allow progression through later stages of meiosis. Fully grown oocytes were treated with progesterone (5 μM) for 3 h, a time where GVBD had occurred in ~20% of the oocytes, and a time when substantial *mos* protein synthesis has occurred (Sagata et al., 1989*a*; Watanabe et al., 1989; data not shown). Oocytes not displaying GVBD at 3 h were injected with either sense or antisense *mos*-specific oligonucleotides. Under these conditions, GVBD was not blocked by *mos* antisense oligonucleotides (Table I). Oocytes were cultured for an additional 8 h to allow progression to metaphase II (Gerhart et al., 1984). When these oocytes were tested for MPF activity, none was found in the oocytes depleted of *mos* RNA (Table I). Collectively, these results and previous findings (Sagata et al., 1988, 1989*a*, *b*; Freeman et al., 1989; Barrett et al., 1990; Paules et al., 1989) show that de novo synthesis of endogenous pp39^{mos} is required after GVBD for mouse or *Xenopus* oocyte maturation and is required for the appearance and/or stabilization of MPF at metaphase II.

The previous experiments showed that pp39^{mos} synthesis was required after GVBD for the maintenance of MPF. It was formally possible that the maturing oocytes entered the second meiosis with an accompanying rise in MPF, only to have MPF levels fall as a function of time in the absence of *mos*. To determine whether the second rise in MPF occurred, oocytes undergoing GVBD after 4 h of progesterone treatment were injected with either sense or antisense *mos* oligonucleotides and crude MPF extracts were prepared at half hour intervals. These extracts were then tested for MPF activity and histone H1 kinase activity (Fig. 1). These analyses demonstrate that MPF and histone H1 kinase activities terminally decrease within the first half hour after *mos* antisense oligonucleotides were injected (Fig. 1), while sense oligonucleotide injected oocytes showed typical cycling of MPF and histone H1 kinase activity. These data show that pp39^{mos} synthesis

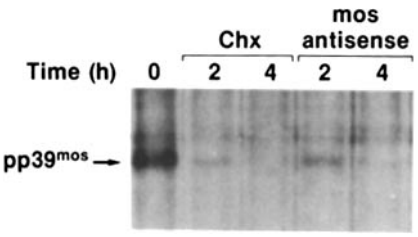

Figure 2. Kinetics of pp39^{mos} proteolytic degradation after either antisense oligonucleotide injection or cycloheximide treatment of oocytes. Stage VI oocytes were continuously labeled during progesterone treatment. After 3 h, oocytes were either injected with antisense oligonucleotides or treated with cycloheximide (10 μg/ml). At the indicated times, groups of 50 oocytes were homogenized in RIPA buffer and immunoprecipitation analysis was performed.

is required for the stabilization of MPF at metaphase I as well as the induction of MPF at metaphase II. To prove that pp39^{mos} was lost after antisense oligonucleotide treatment, as suggested in previous studies with cycloheximide (Watanabe et al., 1989), we continuously labeled oocytes for 3 h in the presence of progesterone before microinjection with antisense oligonucleotides or treatment of cycloheximide. These analyses show that with either treatment the *mos* product disappeared very rapidly (Fig. 2).

Extracts from c-mos^{xe}-transformed NIH/3T3 Cells Contain CSF, but Not MPF

The *mos* product was required for MPF activity during meiotic maturation in oocytes. In mouse cells transformed by c-*mos*^{xe} (Freeman et al., 1989; Yew et al., 1991), *mos* is expressed constitutively, and it has been proposed that this may result in the stabilization of MPF during interphase (Sagata et al., 1989*b*). We tested extracts prepared from NIH/3T3 cells transformed by the *Xenopus mos* product for CSF and MPF activity. Extracts microinjected into one blastomere of a 2-cell embryo prevented cleavage as did control CSF extract prepared from *Xenopus* eggs, while the uninjected blastomeres continued to cleave (Fig. 3). In contrast, extracts prepared from nontransformed NIH/3T3 cells or NIH/3T3 cells transformed by other oncogenes (i.e., the tyrosine kinase oncogene, *tpr-met* [Park et al., 1986], or

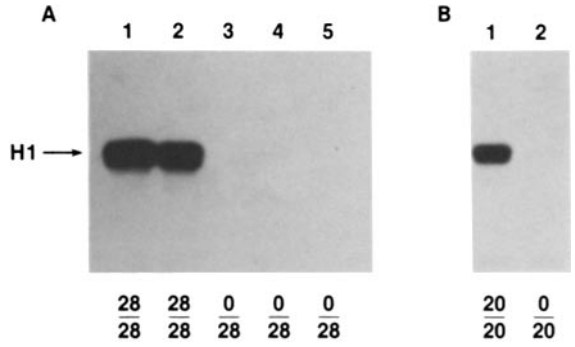

Figure 4. (*A*) MPF and histone H1 kinase activity in extracts from c-*mos*^{xe}-transformed cells. Extracts were prepared in CSF buffer and tested for the ability to phosphorylate histone H1 in vitro as described in Materials and Methods. The samples representing *Xenopus* egg extracts (lane *1*), a mixture of *Xenopus* egg and c-*mos*^{xe}-transformed cell extracts (lane *2*), c-*mos*^{xe}-transformed cell extract (lane *3*), nontransformed NIH/3T3 cell extract (lane *4*), and immature *Xenopus* oocyte extract (lane *5*) were resolved by SDS-PAGE. (*B*) Extracts from nocodazole arrested NIH/3T3 cells (lane *1*) and asynchronously growing NIH/3T3 cells (lane *2*) were prepared and tested for histone H1 kinase activity. 30 nl of the extracts described in *A* and *B* were microinjected into fully grown oocytes maintained in MBS containing cycloheximide (10 μg/ml) as described in Materials and Methods. The number of oocytes that underwent GVBD within 3-h postinjection are indicated above the number of oocytes injected. These numbers are represented below the corresponding lanes in *A* and *B*.

H-*ras*^{val12}) were unable to induce cleavage arrest when injected into embryos. Thus, extracts from somatic cells transformed by the *Xenopus mos* proto-oncogene product specifically induced an embryonic cleavage arrest that was not the result of an activity found in nontransformed NIH/3T3 cells or derivatives of these cells transformed by other oncogenes. CSF arrests embryonic cleavage at metaphase by preventing directly or indirectly the inactivation of MPF (Newport and Kirschner, 1984; Murray et al., 1989; Sagata et al., 1989*b*), and cleaving embryos arrested in metaphase by extracts from c-*mos*^{xe}-transformed cells, like embryos arrested by authentic egg CSF, were positive for MPF activity (data not shown).

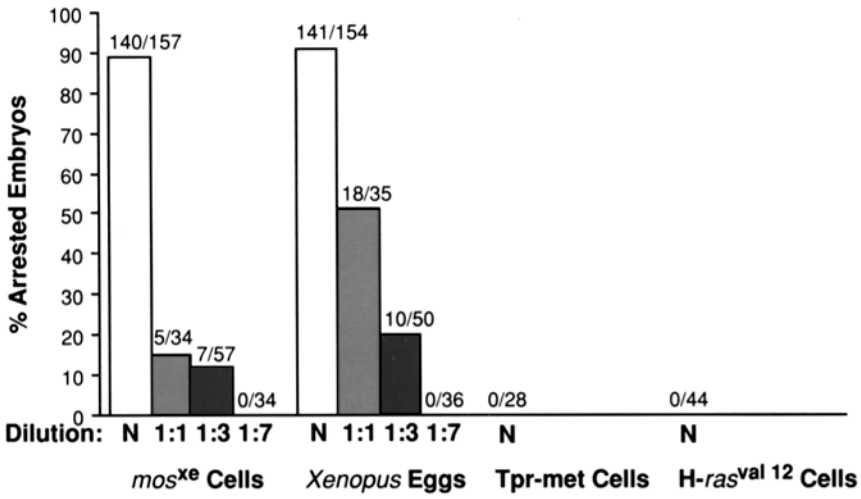

Figure 3. CSF activity in *mos*^{xe}-transformed cells and *Xenopus* eggs. Extracts were prepared from *Xenopus* eggs and NIH/3T3 cells transformed by c-*mos*^{xe}, *tpr-met*, or H-*ras*^{val12}, and 30 nl was injected into 1 cell of a 2-cell embryo as described in Materials and Methods. The indicated dilutions were made in CSF extraction buffer. Embryos were scored for arrest in the 2- to 8-cell stage. The number of arrested embryos per number of embryos injected are indicated above each histogram bar.

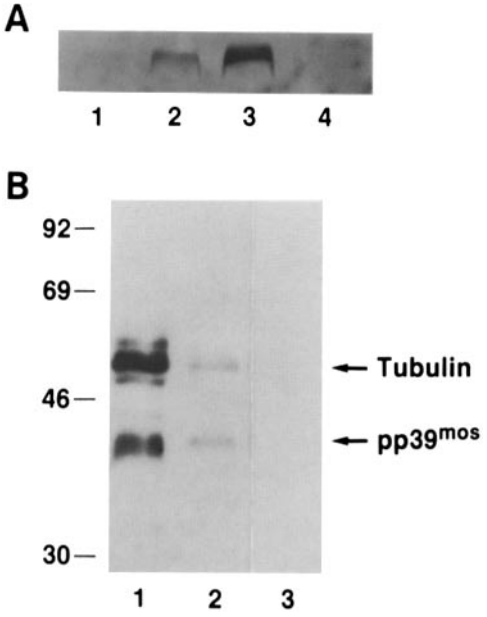

Figure 5. (*A*) Western blot analysis of extracts prepared from *Xenopus* eggs and c-*mos*^{xe}-transformed cells. Immunoprecipitations and Western blot analysis were performed on 100 μl of extract prepared from *Xenopus* eggs (lane *1*), 17 μl from c-*mos*^{xe}-transformed cells (lane *2*), 100 μl from c-*mos*^{xe}-transformed cells (lane *3*), and 100 μl from nontransformed NIH/3T3 cells (lane *4*) as described in Materials and Methods. (*B*) In vitro autophosphorylation of pp39^{mos} from *Xenopus* eggs and c-*mos*^{xe}-transformed cell extracts. 100 μl extracts from *Xenopus* eggs (lane *1*), c-*mos*^{xe}-transformed cells (lane *2*), and nontransformed NIH/3T3 cells (lane *3*) were prepared in CSF buffer, then subjected to immunoprecipitation, in vitro autophosphorylation analysis, and Western transfer as described in Materials and Methods. pp39^{mos} and tubulin are denoted.

The previous experiments demonstrated that CSF activity was present in c-*mos*^{xe}-transformed NIH/3T3 cell extracts. However, these same extracts were negative for MPF activity, while extracts prepared from either *Xenopus* eggs or mitotically arrested NIH/3T3 cells were positive for MPF (Fig. 4). We also tested the c-*mos*^{xe}-transformed cell extracts for MPF-associated histone H1 kinase activity (Fig. 4) and, while extracts from eggs and mitotic NIH/3T3 cells displayed high levels of this enzymatic activity, extracts from c-*mos*^{xe}-transformed cells were no higher in this activity than extracts prepared from interphase nontransformed NIH/3T3 cells (Fig. 4). In addition, there was no evidence for the existence of an MPF inhibitory factor in c-*mos*^{xe}-transformed cell extracts, since mixtures of this extract with a *Xenopus* egg extract were positive for both MPF and histone H1 kinase activity (Fig. 4). Thus, CSF activity is present in interphase *Xenopus mos*-transformed cells, but MPF does not appear to be. We have not yet tested NIH/3T3 cells transformed with other *mos* genes.

The Levels of CSF Activity Are Similar in Eggs and mos^{xe}-transformed Cells

The level of CSF activity observed in extracts from c-*mos*^{xe}-transformed cells was similar to the level found in an equal volume of extracts from unfertilized *Xenopus* eggs, i.e., the egg extract had slightly more activity at a 1:1 dilution (Fig. 3). The CSF activities were similar on a per unit volume basis even though the concentration of *mos* protein was 16 μg/ml in c-*mos*^{xe}-transformed cell extracts and 40 ng/ml in egg extracts. We observed the same results when these extracts were diluted into cytosols prepared from either NIH/3T3 cells or from fully grown oocytes that lack *mos* (data not shown). Thus, the difference in specific activity is not due to transacting inhibitors in the mouse cells or to an apparent need for limiting factors present in the oocyte. Surprisingly, Western analysis showed that there was at least 40 times more pp39^{mos} in the extract prepared from the asynchronous population of c-*mos*^{xe}-transformed cells when compared to an equal volume of egg extract (Fig. 5 *A*). These results revealed a dramatic variation in the ratio of CSF activity to pp39^{mos}. The observed differences in specific activity were apparently not due to M-phase, since extracts from cells treated with nocodozole for 7–20 h displayed only slightly more CSF activity than *Xenopus* egg extracts at a 1:3 dilution (data not shown).

A correlation has been established between *in vitro* autophosphorylation activity and biological activity of the *mos* proto-oncogene (Singh et al., 1986, 1988; Hannik and Donoghue, 1985; Yew et al., 1991). We compared the relative *in vitro* autophosphorylation activity of the *mos* product immunoprecipitated from equal volumes of CSF extracts prepared from either unfertilized eggs or asynchronously growing c-*mos*^{xe}-transformed NIH/3T3 cells. Despite the 40-fold excess of pp39^{mos} in the c-*mos*^{xe}-transformed cell immune complex (Fig. 5 *A*), we observed that pp39^{mos} autophosphorylation activity in this complex was significantly lower than the activity displayed from an equal volume of *Xenopus* egg extract (Fig. 5 *B*). (The phosphorylated proteins observed in the 55–58-kD range are α- and β-tubulin that complex with and are substrates of pp39^{mos} in vitro and perhaps in vivo [Zhou et al., 1991*a*, *b*]). Moreover, pp39^{mos} autophosphorylation in immune complexes prepared from mitotic c-*mos*^{xe}-transformed cells was only two- to five-fold higher than from asynchronous transformed cell populations (data not shown). This is consistent with the increase in autokinase activity during M-phase recently reported for NIH/3T3 cells transformed by v-*mos* (Liu et al., 1990). We conclude that the specific CSF or kinase activity of the *Xenopus mos* product produced in transformed mouse cells is only a fraction of the activity found in *Xenopus* eggs.

Discussion

In *Xenopus*, the *mos* proto-oncogene product is required for MPF activation prior to GVBD and is a component of CSF, an activity responsible for the stabilization of MPF at metaphase II (Sagata et al., 1988, 1989*b*; Watanabe et al., 1989). Metabolic labeling of oocytes shows that the *mos* product is present throughout maturation (Sagata et al., 1988; Watanabe et al., 1989). We have shown that the *mos* product is necessary during *Xenopus* oocyte maturation before and after GVBD. We tested for pp39^{mos} function during meiosis using two approaches. The injection of crude MPF extracts into oocytes depleted of *mos* RNA allowed oocytes to advance through GVBD, albeit at a significantly slower rate than in the presence of endogenous pp39^{mos}. It is curious that MPF-injected oocytes treated with cycloheximide undergo GVBD within minutes of those competent for pro-

tein synthesis (Gerhart et al., 1984), while GVBD in *mos*-depleted oocytes takes 1 h longer. This could be explained by a requirement for de novo synthesis of an inhibitor of MPF during meiosis I, such as $p40^{MO15}$, recently identified by Shuttleworth et al. (1990). Most importantly, $pp39^{mos}$ synthesis was required after GVBD for the reappearance of MPF activity later in maturation. It has previously been shown that after GVBD, protein synthesis is required for completion of meiosis I and the reappearance of MPF that accompanies meiosis II (Gerhart et al., 1984). Thus far, the proteins that must be synthesized during this period have not been identified; for example, cyclin synthesis is not required for the first and second meiosis (Minshull, J., personal communication). Our experiments show that *mos* protein is required after GVBD in *Xenopus* oocytes as well as before GVBD (Sagata et al., 1988), and perhaps throughout meiosis since our experiments indicate that MPF and histone H1 kinase activities decrease precipitously in the presence of *mos* antisense oligonucleotides. We have also shown that the $pp39^{mos}$ synthesized before GVBD does not eliminate the requirement for *mos* product synthesis after GVBD and is similar to results reported for mouse oocytes (Paules et al., 1989; O'Keefe et al., 1989). Our results as well as previous reports indicate that, directly or indirectly, CSF stabilizes MPF (Masui and Clarke, 1979; Newport and Kirschner, 1984; Murray et al., 1989; Sagata et al., 1989*b*). Our experiments, however, do not distinguish between whether *mos* product is required for the formation of an active MPF, whether the MPF is unstable in the absence of *mos* product, or both.

Constitutive expression of the *mos* proto-oncogene product in somatic cells results in transformation (Oskarsson et al., 1980; Blair et al., 1981). We have postulated that expression of *mos* metaphase activity during interphase is responsible for the transformed phenotype. It has also been suggested that the transformed phenotype may be due to CSF stabilizing MPF during interphase. In this report, we demonstrated that CSF activity is present in c-mos^{xe}-transformed cells. However, we were unable to detect MPF or its associated histone H1 kinase activity in extracts prepared from an asynchronous population of the c-mos^{xe}-transformed cells, while these activities were detected in mitotic cells. We conclude that the ratio of CSF to MPF in unfertilized eggs is not maintained in c-mos^{xe}-transformed cells, and the maintenance of significant levels of MPF in these cells is apparently not a component of the transformed phenotype. This is the first example of a biological system showing that CSF activity can exist during interphase and, more importantly, that this activity can persist during interphase and not sustain detectable levels of MPF. It is possible that mouse tissue culture cells may lack factors necessary for responding to CSF and that other products may perform a similar function during mitosis. We believe that the lack of mitotic arrest in mouse cells transformed by the *Xenopus mos* product is not due to species differences since the mouse *mos* product arrests embryonic cleavage as efficiently as the *Xenopus mos* product (Yew et al., 1991). In addition, mouse cells can be efficiently transformed by the mouse *mos* product (Blair et al., 1981; Yew et al., 1991). However, a question is raised regarding the major function of CSF. CSF may stabilize MPF, but only during M-phase, indicating that MPF stability is a function of more than one factor and that CSF stabilization of MPF is not direct and may require the synthesis of other proteins. Consistent with this notion, extracts from c-mos^{xe}-transformed cells which contain active $pp39^{mos}$ do not induce GVBD in the absence of protein synthesis (data not shown). However, this result could be due to the level of *mos* product since more *mos* protein may be required to induce oocyte maturation than embryonic arrest (Sagata et al., 1989*a*, Yew et al., 1991).

The amount of CSF activity per unit volume of cell extract was very similar in extracts prepared from either eggs or c-*mos*-transformed cells. At a 1:1 dilution, *Xenopus* egg extract induced embryonic arrest in approximately three-fold more embryos than that of c-mos^{xe} cell extract and roughly correlates with the five-fold difference in autophosphorylation activity of *mos* product observed in these extracts. Surprisingly, there was a 40-fold excess of *mos* product present in the extract from c-mos^{xe}-transformed cells. However, *mos* protein autophosphorylation is only slightly higher in immune complexes prepared from c-mos^{xe}-transformed cells held in mitosis by nocodozole. Collectively, these results indicate that the *mos* product from transformed cells exhibits only ~2% of the activity found in the product from egg cytosol. CSF extracts diluted into extracts prepared from either NIH/3T3 cells or fully grown *Xenopus* oocytes showed no enhancement of CSF activity, indicating that there were no limiting amounts of enhancing factor(s). Posttranslational modification such as phosphorylation could explain the differences in CSF and c-mos^{xe} autokinase activity. Another possible explanation however, is that the amphibian *mos* product is less stable in mouse cells at 37°C. It is also possible that a nondissociating negative regulatory factor like tubulin oligomers (Zhou et al., 1991) associates with the *mos* product in NIH/3T3 cells, since mixtures of egg and transformed cell extracts showed no reduction in CSF activity (data not shown).

How does a gene product that arrests embryonic cell cleavage transform somatic cells? It has been observed that cells acutely infected with the Moloney murine sarcoma virus express high levels of *mos* product, subsequently round up, and detach from the monolayer (Fischinger and Haapala, 1971; Papkoff et al., 1982). These infected cells express 30- to 100-fold higher levels of *mos* product than transformed cell populations (Papkoff et al., 1982). The morphological alteration of *mos*-infected cells may be similar to that of mitotic rounding and might be an effect of CSF activity. We have proposed that the *mos* product may be responsible for the modification of tubulin and that *mos*, directly or indirectly, is an effector of the transformed phenotype (Zhou et al., 1991*a*, *b*). We have postulated that the transformed phenotype may represent expression of mitotic events during interphase (Sagata et al., 1988) and that selection for the transformed phenotype is a selection for cells expressing levels of $pp39^{mos}$ sufficient for transformation but below the levels required for CSF arrest (Sagata et al., 1989*b*). The specific activity of the *Xenopus* $pp39^{mos}$ is much lower in transformed mouse cells than in unfertilized eggs suggesting that in mouse cells CSF/*mos* function is attenuated. The mechanism of this attenuation may be equivalent to a tumor suppressor function.

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