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

Ira Daar, Richard S. Paules, and George F. Vande Woude

ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, Maryland 21702

**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 pp39mos 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 pp39mos 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 pp39mos 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.

**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 NaHCO3, 10 mM Hepes [pH 7.5], 0.82 mM MgSO4, 0.33 mM Ca(NO3)2, 0.41 mM CaCl2) 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...
Histone HI Kinase Assay

0.3X MMR containing 5% Ficoll 400 for 4-6 h and examined for cleavage. Either eggs or NIH/3T3 cells expressing tpr-met, ras, or bini monoclonal antibody, 10 µg/ml leupeptin, 10 µg/ml aprotinin, 10 µg/ml protein kinase inhibitor). The histone HI kinase reaction was performed by adding 10 µl of the sample to 6 µl of a mixture containing 1 µg of histone HI, 1 µM ATP, and 1 µCi [32P]ATP. The reaction was incubated at room temperature for 15 min and then stopped by the addition of an equal volume of 2X sample buffer (40% SDS, 20% glycerol, 150 mM Tris-HCl [pH 6.8], 0.02% bromophenol blue, 5% 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 In Vitro Kinase Assay

To 100 µl of CSF extract, 5S monoclonal antibody (Sagara et al., 1992a) 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 µg/ml aprotinin 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 µCi 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 MnCl2, 20 µM ATP, 50 µCi of [32P]ATP) the reaction mix was incubated at room temperature for 10 min. 2X 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 either the activated H-ras oncogene (gift from Dr. Youyoung Lu) or the tpr-met oncogene (Park et al., 1990) were also cultured in DMEM with 8% calf serum. NIH/3T3 cells transformed by either the activated H-ras oncogene (gift from Dr. Youyoung Lu) were cultured in DMEM containing 0.4 mg/ml of nocodazole (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 (Sagara et al., 1988). To examine whether pp39mos 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 pp39mos. Oocytes were cultured 8 h following GVBD to allow for progression through meiosis I and arrest at metaphase II (Gerhart et al., 1984). Cytosolic extract was formed by adding 10 µl of the sample to 6 µl of a mixture containing 1 µg of histone HI, 1 µM ATP, and 1 µCi [32P]ATP. The reaction was incubated at room temperature for 15 min and then stopped by the addition of an equal volume of 2X sample buffer (40% SDS, 20% glycerol, 150 mM Tris-HCl [pH 6.8], 0.02% bromophenol blue, 5% 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.

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

| Treatment | Donor | Recipient |
|-----------|-------|-----------|
| * Antisense oligonucleotide plus MPF | 100 | 0 |
| † Progestrone plus antisense oligonucleotide | 100 | 0 |
| Progestrone plus sense oligonucleotide | 100 | 0 |

* 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 cytosol-extracted (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 cytosol-extracted (10 µg/ml) oocytes and scored for GVBD as described in Materials and Methods.

The Journal of Cell Biology, Volume 114, 1991

330
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 pp39mos.

We tested whether pp39mos 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., 1989a; 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, 1989a, b; Freeman et al., 1989; Barrett et al., 1990; Paules et al., 1989) show that de novo synthesis of endogenous pp39mos 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 pp39mos 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 pp39mos synthesis
Figure 2. Kinetics of pp39mos 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 pp39mos 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°-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° (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., 1989b). 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 un.injected 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 H rasVal(12)) were unable to induce cleavage arrest when injected into embryos. Thus, extracts from somatic cells transformed by the Xenopus mos proto-oncogene 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., 1989b), and cleaving embryos arrested in metaphase by extracts from c-mos°-transformed cells, like embryos arrested by authentic egg CSF, were positive for MPF activity (data not shown).

Figure 3. CSF activity in mos°-transformed cells and Xenopus eggs. Extracts were prepared from Xenopus eggs and NIH/3T3 cells transformed by c-mos°, tpr-met, or H rasVal(12), 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 4. (A) MPF and histone H1 kinase activity in extracts from c-mos°-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°-transformed cell extracts (lane 2), c-mos°-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 corresponding lanes in A and B.
Western blot analysis were performed on 100 µl of extract prepared from Xenopus eggs and c-mos-transformed cells. Immunoprecipitations and Western transfer as described in Materials and Methods. (A) Western blot analysis of extracts prepared from Xenopus eggs (lane 1), 17 µl from c-mos-transformed cells (lane 2), 100 µl from c-mos-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 pp39mos from Xenopus eggs and c-mos-transformed cell extracts. 100 µl extracts from Xenopus eggs (lane 1), c-mos-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. pp39mos and tubulin are denoted.

The previous experiments demonstrated that CSF activity was present in c-mos-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-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-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-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-transformed Cells

The level of CSF activity observed in extracts from c-mos-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-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 pp39mos in the extract prepared from the asynchronous population of c-mos-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 pp39mos. The observed differences in specific activity were apparently not due to M-phase, since extracts from cells treated with nocodazole 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-transformed NIH/3T3 cells. Despite the 40-fold excess of pp39mos in the c-mos-transformed cell immune complex (Fig. 5 A), we observed that pp39mos 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 pp39mos in vitro and perhaps in vivo [Zhou et al., 1991a, b]). Moreover, pp39mos autophosphorylation in immune complexes prepared from mitotic c-mos-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, 1989b; 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 pp39mos 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 pp39mos. 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 p40mos, recently identified by Shuttleworth et al. (1990). Most importantly, pp39mos 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 (Minschull, 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 pp39mos 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., 1989b). 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"-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"-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"-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"-transformed cells which contain active pp39mos 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, 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" 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"-transformed cells. However, mos protein autophosphorylation is only slightly higher in immune complexes prepared from c-mos"-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" 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., 1991a, 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 pp39mos sufficient for transformation but below the levels required for CSF arrest (Sagata et al., 1989b). The specific activity of the Xenopus pp39mos 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.

We thank R. Zhou for very helpful discussions and advice, Youyong Lu for the H-ras-transformed cell line, R. Zhou for critical review of the
manuscript, A. Arthur for editorial assistance, and L. Summers for expert assistance in preparing the manuscript.

Research sponsored by the National Cancer Institute, Department of Health and Human Services under contract No. NOI-CO-74101 with ABL. The contents of this publication do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Received for publication 7 December 1990 and in revised form 10 April 1991.

References

Barrett, C. B., R. M. Schroette, F. A. Van der Hoorn, S. K. Nordeen, and J. L. Maller. 1990. Har- 585x1168 activa...in Xenopus oocytes: evidence for c-mos-

Bumette, W. N. 1981. Western blotting: electrophoretic transfer of proteins from SDS-polyacrylamide gels to unmodified nitrocellulose and radio-

egraphic detection of radioiodinated protein. A. Anal. Biochem. 112:195-203.

Cyert, M. S., and M. W. Kirschner. 1988. Regulation of MPF activity in vitro. Cell. 53:185-195.

Draetta, G., F. Luca, J. Westerdorf, L. Brizuela, J. Ruderman, and D. Beach. 1989. cdc2 protein kinase is complexed with both cyclin A and B: evidence for proteolytic inactivation of MPF. Cell. 56:829-838.

Dumont, J. 1972. Oogenesis in Xenopus laevis. Stages of oocyte development in laboratory maintained animals. J. Morphol. 136:153-180.

Dunphy, W. G., L. Brizuela, D. Beach, and J. Newport. 1988. The Xenopus cdc2 protein is a component of MPF, a cytoplasmic regulator of mitosis. Cell. 54:423-431.

Fischinger, P. J., and D. K. Haapala. 1971. Quantitative interaction of lineae with proline and aspartic acid in peptides of the chromosomal DNA. J. Biol. Chem. 246:2543-2549.

Gerhart, J., M. Wu, and M. Kirschner. 1984. Cell cycle dynamics of an M-phase-specific cytoplasmic factor in Xenopus laevis oocytes and eggs. J. Cell Biol. 98:1247-1255.

Gurdon, J. B., and M. P. Wickens. 1983. The use of Xenopus oocytes for the expression of cloned genes. Methods Enzymol. 101:370-387.

Hankin, M., and D. J. Donoghue. 1985. Lysine residue 121 in the proposed ATP-binding site of the v-mos protein is required for transformation. Proc. Natl. Acad. Sci. USA. 82:7894-7898.

Liu, J., B. Singh, D. Wiodek, and R. B. Arlinghaus. 1990. Cell cycle-mediated structural and functional alteration of p85α protein kinase activity. Oncogene. 5:171-178.

Maller, J. L. 1985. Regulation of amphibian oocyte maturation. Cell. Differ. 16:211-221.

Masui, Y., and H. J. Clarke. 1979. Oocyte maturation. Int. Rev. Cytol. 57:185-282.

Masui, Y., and C. L. Markert. 1971. Cytoplasmic control of nuclear behavior during meiotic maturation of frog oocytes. J. Exp. Zool. 117:129-146.

Meijer, L., D. Arion, R. Golsteijn, J. Pines, L. Brizuela, T. Hunt, and D. Beach. 1989. Cyclin is a component of this sea urchin M-phase specific histo-

e H1 kinase. EMBO (Eur. Mol. Biol. Organ.) J. 8:2275-2282.

Miake-Lye, R., J. W. Newport, and M. W. Kirschner. 1983. Maturation promoting factor induces nuclear envelope breakdown in cycloheximide ar-

rested embryos of Xenopus laevis. J. Cell Biol. 97:81-91.

Murray, A. W., M. Solomon, and M. W. Kirschner. 1989. The role of cyclin synthesis and degradation in the control of maturation promoting factor. Na-

ture (Lond.). 339:280-285.

Newport, J. W., and M. W. Kirschner. 1984. Regulation of the cell cycle dur-

ing Xenopus development. Cell. 37:731-742.

O'Keefe, S. J., H. Wolfs, A. A. Kissingi, and G. M. Cooper. 1989. Microinjection of antisense c-mos oligonucleotides prevents meiosis II in the matur-

ing mouse egg. Proc. Natl. Acad. Sci. USA. 86:7038-7042.

Oskarsson, M., W. McClements, D. G. Blair, J. V. Maizel, and G. F. Vande Woude. 1980. Properties of a normal mouse egg DNA sequence (sarc) ho-

mologous to the src sequence of Moloney sarcoma virus. Science (Wash. DC). 207:1222-1224.

Parkoff, J. M., I. M. Verma, and T. Hunter. 1982. Detection of a transforming gene product in cells transformed by Moloney murine sarcoma virus. Cell. 29:417-426.

Park, M., M. Dean, C. S. Cooper, M. Schmidt, S. J. O'Brien, D. G. Blair, and G. F. Vande Woude. 1986. Mechanisms of met oncogene activation. Cell. 45:895-904.

Pantes, R. S., R. Bucione, R. C. Moschel, G. F. Vande Woude, and J. J. Ep-

pig. 1989. Mouse mos protooncogene product is present and functions during oogenesis. Proc. Natl. Acad. Sci. USA. 86:5395-5399.

Pines, J., and T. Hunter. 1989. Isolation of a human cyclin cDNA: evidence for cyclin mRNA and protein regulation in the cell cycle and for interaction with p34cdc2. Cell. 58:833-846.

Papage, N., M. Oskarsson, T. Copeland, J. Brumbaugh, and G. F. Vande Woude. 1988. Function of c-mos proto-oncogene product in meiotic matura-

tion in Xenopus oocytes. Nature (Lond.). 335:519-525.

Sagata, N., I. Daar, M. Oskarsson, S. D. Showalter, and G. F. Vande Woude. 1986a. The product of the mos proto-oncogene as a candidate "initiator" for oocyte maturation. Science (Wash. DC). 245:643-646.

Sagata, N., N. Watanabe, G. F. Vande Woude, and Y. Ikawa. 1989b. The c-mos proto-oncogene product is a cytoskeletal factor responsible for meiotic arrest in vertebrate eggs. Nature (Lond.). 342:512-518.

Shuttleworth, J., R. Godfrey, and A. Colman. 1990. p42cdc2, a cdc2-related protein kinase involved in negative regulation of meiotic maturation of Xeno-

pus oocytes. EMBO (Eur. Mol. Biol. Organ.) J. 9:3233-3240.

Singh, B., M. Hannik, D. J. Donoghue, and R. B. Arlinghaus. 1986. p37 associated serine/threonine protein kinase activity correlates with the cellular transformation function of v-mos. J. Virol. 60:1148-1152.

Singh, B., C. Wittenberg, M. Hannik, S. Reed, D. J. Donoghue, and R. B. Arlinghaus. 1988. The histidine-221 to tyrosine substitution in v-mos abolishes its biological function and its protein kinase activity. Virology. 164:114-120.

Wasserman, W. J., and Y. Masui. 1975. Effects of cycloheximide on a cytoplastic factor initiating meiotic maturation in Xenopus oocytes. Exp. Cell Res. 91:381-388.

Watanabe, N., G. F. Vande Woude, Y. Ikawa, and N. Sagata. 1989. Specific proteolysis of the c-mos proto-oncogene product by calpain on fertilization of Xenopus eggs. Nature (Lond.). 342:505-511.

Yew, N., M. Oskarsson, J. Daar, D. G. Blair, and G. F. Vande Woude. 1991a. Mos gene transforming efficiencies correlate with oocyte maturation and cytoskeletal factor activities. Mol. Cell. Biol. 11:604-610.

Zhou, R., M. Oskarsson, R. Paules, N. Schultiz, D. Cleveland, and G. F. Vande Woude. 1991b. The ability of the c-mos product, pp39mos, to associate with and phosphorylate tubulin. Science (Wash. DC). 251:671-675.

Zhou, R., S. Rulong, P. Pintdo, S. G. Vande Woude. 1991b. In vitro and in vivo characterization of p39mos association with tubulin. Cell Growth Diff. 2:257-265.