Effects of the v-*mos* Oncogene on *Xenopus* Development: Meiotic Induction in Oocytes and Mitotic Arrest in Cleaving Embryos

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Abstract. Previous work has demonstrated that the *Xenopus* protooncogene *mos*^{xe} can induce the maturation of prophase-arrested *Xenopus* oocytes. Recently, we showed that *mos*^{xe} can transform murine NIH3T3 fibroblasts, although it exhibited only 1–2% of the transforming activity of the v-*mos* oncogene. In this study we have investigated the ability of the v-*mos* protein to substitute for the *mos*^{xe} protein in stimulating *Xenopus* oocytes to complete meiosis. Microinjection of in vitro synthesized RNAs encoding either the *mos*^{xe} or v-*mos* proteins stimulates resting oocytes to undergo germinal vesicle breakdown. Microinjection of an antisense oligonucleotide spanning the initiation codon of the *mos*^{xe} gene blocked progesterone-induced oocyte maturation. When oocytes were microinjected first with the *mos*^{xe} antisense oligonucleotide, and subsequently with in vitro synthesized v-*mos* RNA, meiotic maturation was rescued as evidenced by germinal vesicle breakdown. The v-*mos* protein exhibited in vitro kinase activity when recovered by immunoprecipitation from either microinjected *Xenopus* oocytes or transfected monkey COS-1 cells; however, in parallel experiments, we were unable to detect in vitro kinase activity associated with the *mos*^{xe} protein. Microinjection of in vitro synthesized v-*mos* RNA into cleaving *Xenopus* embryos resulted in mitotic arrest, demonstrating that the v-*mos* protein can function like the *mos*^{xe} protein as a component of cytostatic factor. These results exemplify the apparently conflicting effects of the v-*mos* protein, namely, its ability to induce maturation of oocytes, its ability to arrest mitotic cleavage of *Xenopus* embryo, and its ability to transform mammalian fibroblasts.

THE v-*mos* oncogene, derived from the acute transforming retrovirus Moloney murine sarcoma virus, encodes a serine/threonine protein kinase localized in the cytoplasm of transformed fibroblasts (Maxwell and Arlinghaus, 1985*a*; Papkoff et al., 1983). However, the identity of the substrate(s) recognized by v-*mos* remains conjectural and the biochemical mechanism whereby v-*mos* transforms is still a mystery. In a variety of organisms, significant expression of the cellular homolog, c-*mos*, appears only in germ cells with little, if any, detectable expression in somatic cells (reviewed in Propst et al., 1988). Recently, it was demonstrated that expression of the *Xenopus* c-*mos* gene (*mos*^{xe}) is required for the maturation of *Xenopus* oocytes (Sagata et al., 1988). Microinjection of oocytes with *mos*^{xe}-specific antisense oligonucleotides prevents hormone-induced germinal vesicle breakdown (GVBD).[1] Moreover, prophase-arrested oocytes can be induced to undergo GVBD by microinjection of in vitro transcribed *mos*^{xe} RNA, demonstrating that expression of the *mos*^{xe} protein is sufficient for reinitiation of normal meiosis (Freeman et al., 1989; Sagata et al., 1989*a*). *mos*^{xe} RNA is present during oocyte growth and maturation and persists in the developing embryo through blastulation; however, the *mos*^{xe} protein is only detected during hormone-induced oocyte maturation and is rapidly degraded shortly after fertilization (Watanabe et al., 1989).

Somewhat different results have been reported using mouse oocytes, where microinjection of murine c-*mos*-specific antisense oligonucleotides fails to block GVBD but does prevent extrusion of the first polar body (Paules et al., 1989) or, in other experiments, the initiation of meiosis II (O'Keefe et al., 1989). The murine c-*mos* protein is present in oocytes before maturation, suggesting that this store of c-*mos* protein may stimulate GVBD even when de novo synthesis of c-*mos* protein is prevented by microinjection of antisense oligonucleotides. Thus, in both *Xenopus* and mouse oocytes, the microinjection of *mos*-specific antisense oligonucleotides blocks the completion of meiosis at the first point where de novo translation appears to be required.

In other experiments, microinjection of *Xenopus* oocytes with *mos*^{xe}-specific antisense oligonucleotides inhibited GVBD induced by injection of the p21^{ras} protein (Barrett et al., 1990). Hormone-induced maturation of oocytes is known to stimulate two kinase activities: one which phosphorylates

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

the 40S ribosomal subunit protein S6 (Nielsen et al., 1982), and another which phosphorylates histone H1 (Cicirelli et al., 1988). The activation of both of these kinase activities by progesterone or insulin treatment is blocked by mos^{xe} antisense oligonucleotides (Barrett et al., 1990). Histone H1 kinase activity has recently been attributed to the *cdc*2 protein (Arion et al., 1988), the catalytic subunit of maturation-promoting factor (MPF) (Dunphy et al., 1988; Gautier et al., 1988). MPF is a cell cycle-regulated protein complex that induces oocyte maturation and, more generally, controls entry into mitosis (Gerhart et al., 1984; Dunphy and Newport, 1988). In light of these results, it seems likely that mos^{xe} is involved in the activation or stabilization of MPF.

A second function for the mos^{xe} protein has been suggested based on experiments in which mos^{xe} RNA was microinjected into cleaving *Xenopus* embryos (Sagata et al., 1989*b*). Cytostatic factor (CSF) has been characterized as an activity present in extracts from unfertilized eggs that maintains the unfertilized egg in a state of meiotic arrest, possibly by stabilizing MPF (Masui and Markert, 1971). Injection of mos^{xe} RNA into one blastomere of a two-cell embryo resulted in mitotic cleavage arrest of the injected blastomere, identical to the results observed with CSF-containing extracts. In addition, neutralization or immunodepletion of the mos^{xe} protein from egg extracts with mos^{xe} may arrest mitotic cleavage by preventing the decrease of MPF activity which accompanies the normal cell cycle, by either directly or indirectly stabilizing MPF.

The c-*mos* genes from a variety of species can transform mouse fibroblasts in vitro (Blair et al., 1981; van der Hoorn et al., 1982; Blair et al., 1986; Schmidt et al., 1988; Paules et al., 1988; Freeman et al., 1989). However, transformation by the v-*mos* gene is 50–100 times more efficient than transformation induced by mos^{xe}. In addition to its transforming activity, the v-*mos* protein possesses an intrinsic protein kinase activity demonstrated by in vitro autophosphorylation (Maxwell and Arlinghaus, 1985*a*). The results discussed above raise the possibility that transformation by v-*mos* may be a consequence of the inappropriate expression of a protein kinase which acts to stabilize MPF activity.

In this study, we have undertaken a direct comparison between v-*mos* and mos^{xe} with regard to their ability to function in *Xenopus* oocytes. Microinjection of oocytes with v-*mos* RNA induced GVBD in a manner analogous to that of mos^{xe}. Induction of GVBD by v-*mos* was not affected by preinjection of the oocytes with mos^{xe}-specific antisense oligonucleotides. We also demonstrate that the v-*mos* protein expressed in oocytes or in mammalian tissue culture cells possesses significantly greater kinase activity than the mos^{xe} protein. Finally, we show that v-*mos* can function to arrest cleaving *Xenopus* embryos and thus can substitute for the mos^{xe} protein as a component of CSF.

Materials and Methods

In Vitro Transcription of RNA

The wild type mos^{xe}/pSP64(polyA) plasmid and the mutant $mos^{xe(R90)}$ gene, with the codon for lysine-90 replaced with a codon for arginine, have been described previously (Freeman et al., 1989). A similar plasmid was constructed to allow for expression of the v-*mos* gene under the transcriptional control of the SP6 promoter. A Bam HI fragment containing the complete v-*mos* coding region from strain 124 of Moloney murine sarcoma virus (Van Beveren et al., 1981) was inserted into pSP64(polyA) (Promega Biotec, Madison, WI) upstream of the synthetic poly(A) tract. The v-*mos*, mos^{xe}, and $mos^{xe(R90)}$ plasmids were then linearized at a unique restriction enzyme site downstream of the poly(A) tract and used as templates for transcription of 5'-capped and polyadenylated RNAs by SP6 RNA polymerase (Promega Biotec) as described (Melton, 1987). The integrity of the RNAs used in microinjections was determined by electrophoresis through 1.5% agarose/2.2 M formaldehyde gels and by in vitro translation in rabbit reticulocyte lysates (Amersham Corp., Arlington Heights, IL) containing 50 μCi of [^{35}S]methionine.

Microinjection of RNA into Xenopus Oocytes and Embryos

Stage VI oocytes were manually dissected from ovaries surgically removed from female *Xenopus* (Xenopus I, Ann Arbor, MI). After overnight incubation at 18°C in modified Barth's solution, MBS-H (88 mM NaCl, 1 mM KCl, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 2.4 mM $NaHCO_3$, 10 mM Hepes pH 7.4, 0.1 mg/ml each of penicillin and streptomycin), healthy oocytes were microinjected with 50 nl of in vitro transcribed RNA. Microinjected oocytes were incubated in MBS-H at room temperature and scored for GVBD by the appearance of a white spot in the pigmented animal pole (Merriam, 1971). Oocytes were then fixed in 5% trichloroacetic acid and manually dissected to confirm GVBD. As a positive control for GVBD, oocytes were treated with 15 μM progesterone in MBS-H and analyzed for GVBD as above.

Ovulation was induced in animals by injecting with 100 U of pregnant mare serum gonadotropin (Calbiochem-Behring Corp., La Jolla, CA) 3–10 d before injection of 500 U human chorionic gonadotropin (Sigma Chemical Co., St. Louis, MO). Ovulated eggs were collected into MMR solution (5 mM Hepes, pH 7.8, 100 mM NaCl, 2 mM KCl, 1 mM $MgSO_4$, 2 mM $CaCl_2$, 0.1 mM EDTA) and fertilized in vitro. The in vitro fertilized eggs were dejellied in 2% cysteine and cultured in MMR containing 5% Ficoll (Newport and Kirschner, 1982). Two-cell embryos were microinjected in the animal pole of one blastomere with 30 nl of RNA (1 mg/ml) just before completion of the first cleavage.

Microinjection of Antisense Oligonucleotides

An antisense oligonucleotide that spans the mos^{xe} start codon and is complementary to the nucleotide sequence (−)18 to 7 of the mos^{xe} gene was synthesized on an Applied Biosystems 381A DNA synthesizer and purified by chromatography on an oligonucleotide purification cartridge (Applied Biosystems, Inc., Foster City, CA). 50 nl of the oligonucleotide (2 mg/ml) were microinjected into stage VI oocytes. The injected oocytes were incubated in MBS-H at room temperature for 4 h before incubation in 15 μM progesterone in MBS-H or a second microinjection with 50 nl of v-*mos* RNA (2 mg/ml). Oocytes were scored for GVBD as described above.

Immunoprecipitation and In Vitro Kinase Assay of mos^{xe} and v-mos Expressed in Oocytes

Microinjected oocytes were labeled by incubation in MBS-H containing 0.5 mCi/ml each of [^{35}S]cysteine and [^{35}S]methionine for 12 h. Oocytes were rinsed twice in MBS-H and lysed in 2–3 μl per oocyte of Tris/NP-40 buffer (10 mM Tris HCl, pH 6.8, 5 mM EDTA, 150 mM NaCl, 1% NP-40, 10 μg/ml aprotinin (Sigma Chemical Co.) containing 1 mM PMSF and 2 mM DTT. Lysates were centrifuged for 5 min at 10,000 *g* at 4°C to pellet the yolk. The [^{35}S]-labeled supernatants were diluted with 800 μl RIPA buffer (10 mM sodium phosphate pH 7.2, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS, 10 μg/ml aprotinin) containing 1 mM PMSF and immunoprecipitated with either anti(37-55)-serum (Gallick et al., 1985), which recognizes the NH_2-termini of both v-*mos* and mos^{xe}, or anti(mos^{xe})-serum, a rabbit antiserum raised against a peptide (KESNAPPPLGTGL) corresponding to the COOH-terminal 12 amino acids of mos^{xe} crosslinked to BSA. In some cases, the antipeptide sera were preincubated with the appropriate peptide antigen before being added to the lysates. The immunoprecipitates were collected with fixed *Staphylococcus aureus* bacteria (Boehringer Mannheim Biochemicals, Indianapolis, IN) and analyzed by 15% SDS-PAGE and fluorography.

For kinase assays, unlabeled microinjected oocytes were lysed as described above but the supernatants were diluted with 800 μl Tris/NP-40 buffer containing 1 mM PMSF and 2 mM DTT before immunoprecipitation. The immune complexes were collected with fixed *S. aureus* bacteria and in vitro kinase assays were performed as described previously (Maxwell

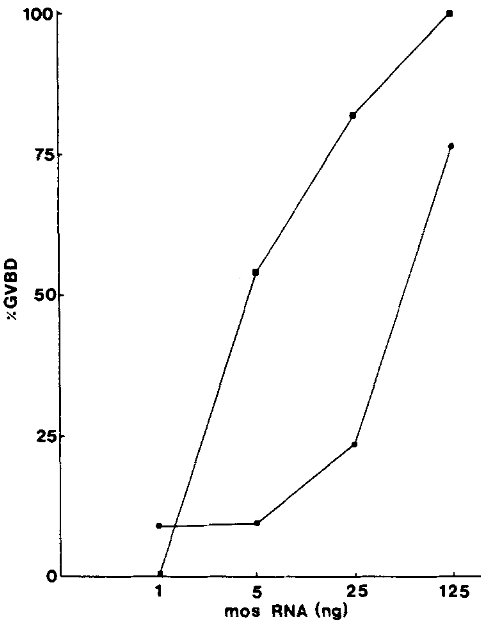

Figure 1. Dose-dependent induction of GVBD by v-*mos*. Healthy stage VI oocytes were microinjected with 50 nl of either v-*mos* or *mos*^{xe} RNAs at concentrations of 20 μg/ml, 0.1 mg/ml, 0.5 mg/ml, and 2.5 mg/ml. Oocytes were cultured in MBS-H for 12 h and scored for the percentage with GVBD as described in Materials and Methods. Groups of 25–30 oocytes were injected for each data point. The percentage GVBD for each point was normalized to the maximum percentage GVBD obtained. Additional experiments confirmed the relationship between v-*mos* and *mos*^{xe} shown above, however, the absolute values for the maximum percentage GVBD observed ranged from 50 to 100% (■) v-*mos*-injected oocytes; (●) *mos*^{xe}-injected oocytes.

and Arlinghaus, 1985*a*; Singh et al., 1988). The phosphorylated proteins were analyzed by 15% SDS-PAGE and autoradiography.

Expression of mos^{xe} *and v-mos in COS-1 Cells*

The v-*mos* and *mos*^{xe} genes were inserted into SV-40 late expression vectors, pJC119 (Sprague et al., 1983) and pMH189, as Xho I and Xho I to Cla I fragments, respectively. pMH189 is a derivative of pJC119 derived by the insertion of Xho I and Cla I linkers into the unique Xho I restriction site of pJC119. COS-1 cells were transfected by the DEAE-dextran method as described previously (Hannink et al., 1986). The cells were labeled for 2 h, 48 h after transfection, with 0.2 mCi/ml each of [^{35}S]cysteine and [^{35}S]methionine in DME lacking cysteine and methionine. Cells were lysed in RIPA buffer and immunoprecipitated with either anti (37–55)-serum or anti(*mos*^{xe})-serum. The immunoprecipitates were collected with fixed *S. aureus* bacteria and analyzed by 15% SDS/PAGE and fluorography.

For in vitro kinase assays, COS-1 cells were transfected with the v-*mos* and *mos*^{xe} genes as described above and lysed in Tris/NP-40 buffer containing 2 mM DTT. Cell lysates were immunoprecipitated and subjected to immune complex kinase assays as described previously (Maxwell and Arlinghaus, 1985*a*; Singh et al., 1988). The phosphorylated proteins were analyzed by 15% SDS-PAGE and autoradiography.

Results

Microinjection of v-mos RNA Induces GVBD in Xenopus Oocytes

Previous work has demonstrated that microinjection of *mos*^{xe} RNA into prophase-arrested *Xenopus* oocytes induces oocyte maturation, characterized by GVBD, in a dose-dependent manner (Freeman et al., 1989; Sagata et al., 1989*a*). To determine if the v-*mos* oncogene is likewise able to induce meiotic progression, we prepared in vitro synthesized v-*mos* RNA for microinjection into oocytes. The v-*mos* coding region was cloned into pSP64 poly(A), and 5′-capped and polyadenylated RNA was transcribed in vitro as described in Materials and Methods. Increasing amounts of either v-*mos* or *mos*^{xe} RNA were microinjected into stage VI oocytes, and 12–14 h later the oocytes were examined for signs of GVBD. The resulting dose-response curve (Fig. 1) shows that v-*mos* RNA was able to induce GVBD over the same range of concentrations as *mos*^{xe} RNA. At the lowest and highest concentrations tested, both v-*mos* and *mos*^{xe} RNAs induced GVBD with roughly comparable efficiencies; however, at intermediate concentrations v-*mos* RNA was 4–6-fold more effective than *mos*^{xe} RNA. We did not observe any differences in the kinetics of GVBD in oocytes injected with v-*mos* RNA compared to those injected with *mos*^{xe} RNA. Although the amount of *mos* RNA injected in these experiments corresponds to a significant fraction of the total polyadenylated RNA in an oocyte, the newly translated *mos* protein represents only a small percentage of the total protein synthesized (data not shown). This may be due to the regulation of protein synthesis in oocytes by some component of the translational machinery other than the level of injected RNA (Laskey et al., 1977; Audet et al., 1987). These results demonstrate that microinjection of v-*mos* RNA induces meiotic progression in *Xenopus* oocytes and suggest that v-*mos* may be able to substitute for *mos*^{xe} in this capacity.

Antisense Inhibition of mos^{xe} *Does Not Prevent Induction of GVBD by v-mos*

Conceivably, oocyte maturation in response to microinjection of v-*mos* RNA might not result directly from the translated v-*mos* protein. For example, the presence of v-*mos* protein in microinjected oocytes might stimulate the synthesis of endogenous *mos*^{xe} protein to a level sufficient to promote GVBD. To directly test this possibility, we carried out double microinjection experiments in which we first microinjected *mos*^{xe} antisense oligonucleotides, which can inhibit hormone-induced maturation of *Xenopus* oocytes by preventing *mos*^{xe} translation (Sagata et al., 1988), and subsequently microinjected v-*mos* RNA in an effort to overcome the inhibition of translation of *mos*^{xe}. For the first microinjection, oocytes were injected with an antisense oligonucleotide that spans the initiator methionine codon for *mos*^{xe}. After incubation in buffer for 4 h, the preinjected oocytes were either treated with progesterone or microinjected with v-*mos* RNA. When oocytes were injected with the antisense oligonucleotide and then treated with progesterone, GVBD occurred in <10% of the oocytes demonstrating the efficacy of the antisense oligonucleotide (Fig. 2). When the preinjected oocytes were injected with v-*mos* RNA, 84% of the oocytes underwent GVBD. This result was very similar to the results observed for either progesterone-treated oocytes or oocytes injected with only v-*mos* RNA. SDS-PAGE analysis of immunoprecipitates of [^{35}S]methionine-labeled oocytes that had been injected with either v-*mos* RNA alone, or with the antisense oligonucleotide followed by v-*mos* RNA, demonstrated that an equal amount of v-*mos* protein was synthe-

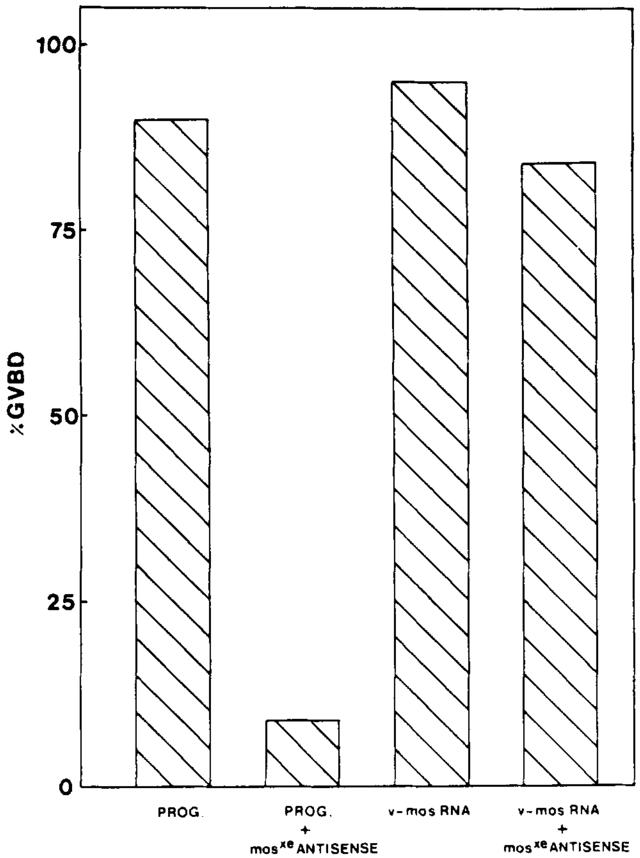

Figure 2. Induction of GVBD by v-*mos* in the presence of *mos*^{xe} antisense oligonucleotides. Groups of 20–25 oocytes were treated either by (*a*) incubation in 15 µM progesterone; (*b*) microinjection with the *mos*^{xe} antisense oligonucleotide followed 4 h later by incubation in 15 µM progesterone; (*c*) microinjection with v-*mos* RNA; or (*d*) microinjection with the *mos*^{xe} antisense oligonucleotide followed 4 h later by microinjection with v-*mos* RNA. Microinjections and scoring of oocytes for GVBD were performed as described in Materials and Methods.

sized in each case (data not shown). These data show that v-*mos* can induce GVBD in oocytes that are deficient in *mos*^{xe} translation and suggest that induction of GVBD by v-*mos* occurs independently of *mos*^{xe}.

The v-mos Protein Exhibits Significantly Greater In Vitro Kinase Activity than the mos^{xe} Protein

The v-*mos* protein, when expressed in mammalian tissue culture cells or in yeast, possesses an intrinsic protein kinase activity evident in in vitro autophosphorylation assays (Maxwell and Arlinghaus, 1985*a*; Singh et al., 1986*b*). To compare the potential in vitro kinase activity of the *mos*^{xe} protein with that of the v-*mos* protein, we performed immune complex kinase assays on oocytes microinjected with v-*mos* or *mos*^{xe} RNA using two different *mos* antisera: anti(37–55)-serum (Gallick et al., 1985), which recognizes an epitope at the NH$_2$-terminus of both v-*mos* and *mos*^{xe}, and anti(*mos*^{xe})-serum, which was raised against a COOH-terminal peptide of *mos*^{xe}. Half of the injected oocytes were labeled with [^{35}S]methionine and, after the occurrence of GVBD, lysates were prepared and subjected to immunoprecipitation with each of the two *mos* antisera (Fig. 3). Progesterone-treated oocytes were also [^{35}S]methionine labeled and immunoprecipitated with anti(37–55)-serum for comparison (lane *1*). Immunoprecipitation of the 41-kD *mos*^{xe} protein and 39-kD v-*mos* protein was specifically blocked by preincubation of the antisera with the cognate peptide antigens as shown in the + lanes. As expected, the COOH-terminal anti(*mos*^{xe})-serum did not immunoprecipitate any v-*mos* protein (lane *9*). Approximately 16 times more *mos*^{xe} protein was synthesized in injected oocytes than in progesterone-treated oocytes. This data, along with the data presented in Fig. 1, suggest that the amount of *mos*^{xe} protein present in the injected oocytes is not directly proportional to the extent of GVBD. This could be explained if synthesis of another protein was limiting the extent of GVBD induced by *mos*.

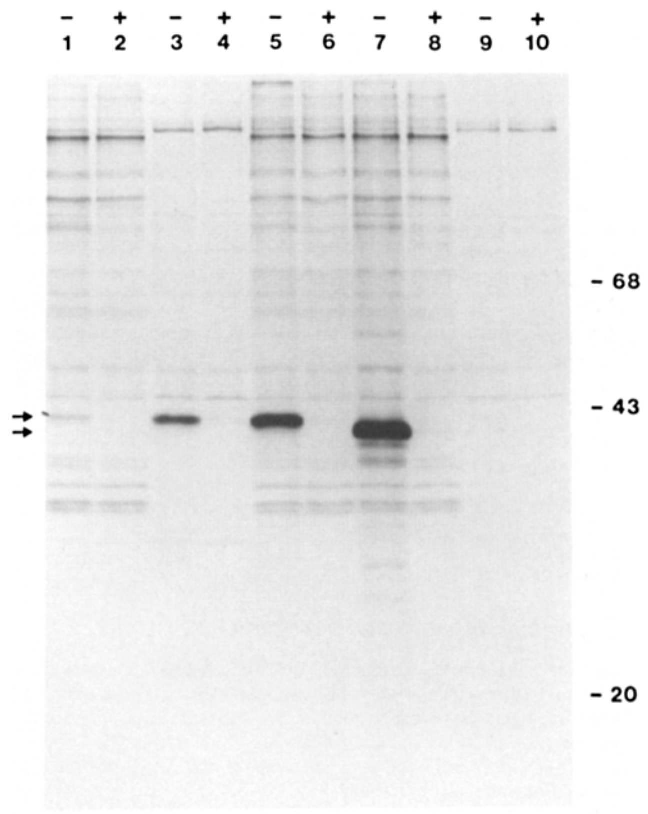

Figure 3. Expression of v-*mos* and *mos*^{xe} in microinjected oocytes. Oocytes were microinjected with 50 nl of 2 mg/ml of v-*mos* or *mos*^{xe} RNA as described in Materials and Methods. After the injections, oocytes were cultured for 12 h in MBS-H containing 500 µCi/ml each of [^{35}S]cysteine and [^{35}S]methionine. Progesterone-treated oocytes were cultured as above except that the media also contained 15 µM progesterone. Each lane represents the immunoprecipitated protein from 10 oocytes. The –/+ indicates whether the immunoprecipitation was performed in the absence or presence of the competing peptide antigen. Immunoprecipitates were analyzed by SDS-PAGE and fluorography for 24 h. Lanes *1* and *2* are immunoprecipitates of progesterone-treated oocytes, lanes *3–6* are from *mos*^{xe}-injected oocytes, and lanes *7–10* are from v-*mos*-injected oocytes. Cell lysates were immunoprecipitated with anti(37–55)-serum, which recognizes both v-*mos* and *mos*^{xe}, shown in lanes *1*, *2*, and *5–8*; or with anti(*mos*^{xe})-serum, lanes *3*, *4*, *9*, and *10*. The arrows indicate the positions of the *mos*^{xe} (*upper arrow*) and the v-*mos* proteins (*lower arrow*). Molecular weight markers are given on the right side of the fluorograph.

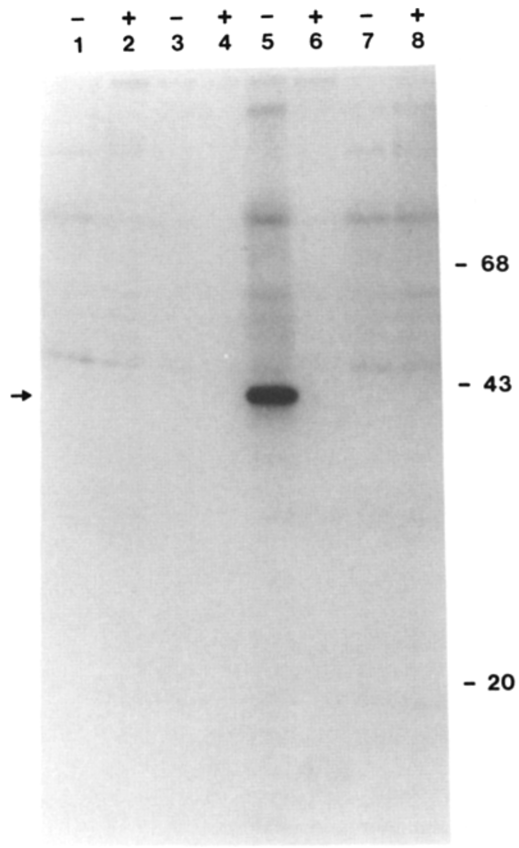

Figure 4. In vitro kinase assay of v-*mos* and *mos*^{xe} expressed in oocytes. Oocytes were microinjected with v-*mos* and *mos*^{xe} RNA as described in Fig. 3 legend. After the microinjections, oocytes were incubated in MBS-H for 12 h, and then lysed and immunoprecipitated as described in Materials and Methods. Immunoprecipitates were formed in the absence (–) or presence (+) of the competing peptide. The immune complexes were then subjected to an in vitro kinase assay using γ-[^{32}P]ATP. Phosphorylated proteins were analyzed by SDS-PAGE and autoradiography. The gel was exposed to film for 3 d with an intensifying screen. Lanes *1–4* are immunoprecipitates from *mos*^{xe}-injected oocytes and lanes *5–8* are from v-*mos*-injected oocytes. Immunoprecipitations were performed with anti(*mos*^{xe})-serum, shown in lanes *1*, *2*, *7*, and *8*; or with anti(37–55)-serum, which recognizes the NH$_2$-termini of both v-*mos* and *mos*^{xe}, lanes *3–6*. The arrow indicates the position of the phosphorylated v-*mos* protein. Molecular weight markers are shown on the right side of the autoradiograph.

The remaining unlabeled oocytes were immunoprecipitated with the two *mos* antisera and subjected to an immune complex kinase assay as described in Materials and Methods (Fig. 4). Only the v-*mos* protein immunoprecipitated with anti(37–55)-serum was specifically phosphorylated. We did not observe phosphorylation of the *mos*^{xe} protein when either of the *mos* antisera were used for the immunoprecipitation. Although the amount of v-*mos* protein synthesized in this experiment was ∼1.8-fold higher than that of the *mos*^{xe} protein (Fig. 3, lanes *5* and *7*), phosphorylation of the v-*mos* protein was ∼110 times that of the background level in the corresponding region of the *mos*^{xe} lanes (estimated by scanning laser densitometry). These results clearly show that the v-*mos* protein expressed in *Xenopus* oocytes can be phosphorylated in vitro and that under comparable conditions, the *mos*^{xe} protein possesses very little, if any, in vitro kinase activity.

To examine the difference in kinase activity between the v-*mos* and *mos*^{xe} proteins further, the two proteins were expressed transiently in monkey COS-1 cells under the transcriptional control of the SV-40 late promoter. Immunoprecipitation of [^{35}S]methionine-labeled lysates prepared from v-*mos*- or *mos*^{xe}-transfected cells revealed that an approximately equal amount of each protein was synthesized (Fig. 5 *A*). However, only the v-*mos* protein showed significant phosphorylation in immune complex kinase assays (Fig. 5 *B*). The *mos*^{xe} protein immunoprecipitated with either of the two *mos* antisera was not phosphorylated in these experiments (Fig. 5 *B* and data not shown), consistent with the results obtained using *mos*^{xe} RNA injected oocytes described above. Thus, the v-*mos* and *mos*^{xe} proteins differ markedly in their ability to be phosphorylated in vitro yet

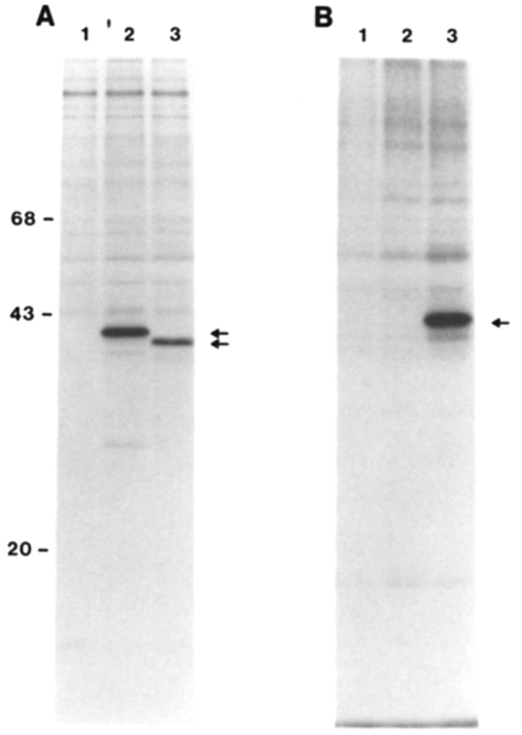

Figure 5 (*A*) Transient expression of v-*mos* and *mos*^{xe} in COS-1 cells. The v-*mos* and the *mos*^{xe} genes were inserted into an SV-40 late expression vector and transfected into COS-1 cells as outlined in Materials and Methods. The cells were labeled for 2 h with [^{35}S]cysteine and [^{35}S]methionine. Cell lysates were subjected to immunoprecipitation with anti(37–55)-serum and resolved by SDS-PAGE followed by fluorography for 3 d. (lane *1*) Mock transfection; (lane *2*) *mos*^{xe} protein; (lane *3*), v-*mos* protein. The arrows indicate the positions of the *mos*^{xe} (*upper arrow*) and the v-*mos* proteins (*lower arrow*). (*B*) In vitro kinase assay. Cell lysates were prepared from COS-1 cells transfected with the v-*mos* or *mos*^{xe} genes. The lysates were immunoprecipitated with anti(37–55)-serum and subjected to an immune complex kinase assay. The phosphorylated proteins were resolved by SDS-PAGE. Autoradiography was performed for 17 h with an intensifying screen. (lane *1*) Mock transfection; (lane *2*) *mos*^{xe} transfection; (lane *3*) v-*mos* transfection. The arrow indicates the position of the v-*mos* protein. The positions of molecular weight markers are shown on the left side of the fluorograph in *A*.

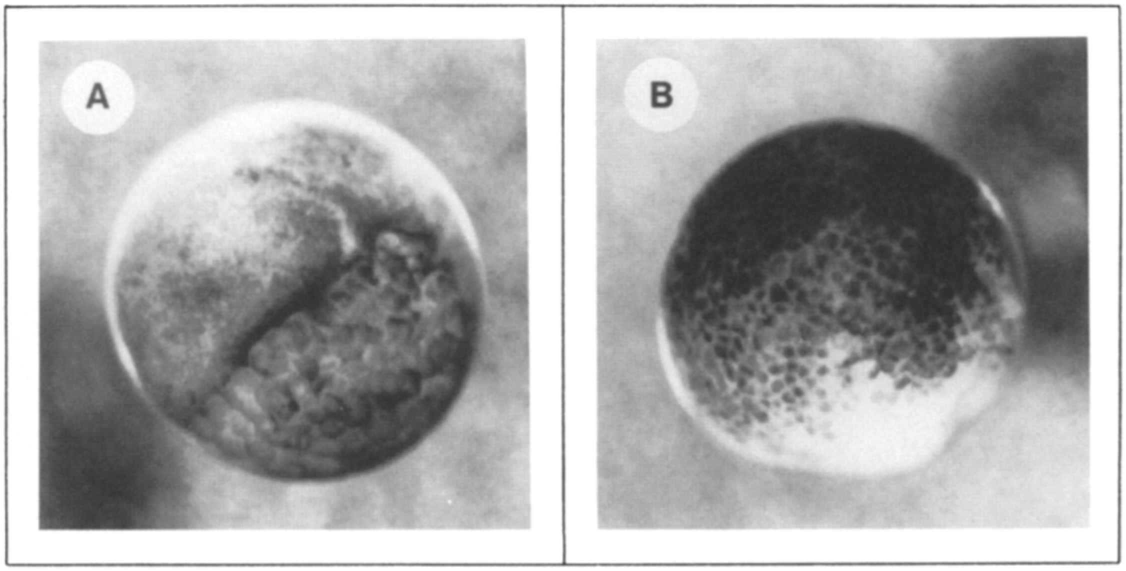

Figure 6. Cleavage arrest of *Xenopus* embryos by v-*mos*. One blastomere of a two-cell embryo was microinjected with either v-*mos* RNA (*A*) or $mos^{xe(R90)}$ RNA (*B*) as described in Materials and Methods. The embryos were photographed (25×) 4 h after injection.

they exert similar biological activities when expressed in oocytes.

Microinjection of v-mos RNA Causes Mitotic Arrest in Xenopus Embryos

Recently, the mos^{xe} protein was shown to function not only as an inducer of oocyte maturation, but also as a component of CSF, an activity found in unfertilized *Xenopus* eggs that is believed to be responsible for maintaining meiotic arrest at metaphase II (Sagata et al., 1989*b*). CSF activity is detected by its ability to induce mitotic arrest when extracts from unfertilized eggs are injected into cleaving embryos (Masui and Markert, 1971). When mos^{xe} RNA is injected into one blastomere of a two-cell embryo, mitotic cleavage is arrested in the injected blastomere while the uninjected half continues to divide normally. Thus, we wished to examine whether v-*mos*, like mos^{xe}, is able to induce mitotic arrest in *Xenopus* embryos.

To test whether v-*mos* can function in place of mos^{xe} as a component of CSF, we microinjected v-*mos* RNA into one blastomere of a two-cell embryo. Just before completion of the first cleavage, the embryos were microinjected with either mos^{xe} RNA, v-*mos* RNA, or RNA synthesized from the $mos^{xe(R90)}$ gene (Freeman et al., 1989) which encodes a point mutation in the canonical ATP-binding site. Microinjection of 30 ng of mos^{xe} RNA into embryos resulted in cleavage arrest, usually before the start of the first or second cleavage of the injected blastomere (data not shown). Interestingly, cleavage of blastomeres injected with 30 ng of v-*mos* RNA was arrested at the same stage as for those injected with 30 ng of mos^{xe} RNA (Fig. 6). However, microinjection of $mos^{xe(90)}$ RNA did not inhibit cleavage of the embryo. Staining of the v-*mos*-arrested embryos with a fluorescent DNA-binding dye revealed that the arrested blastomeres contained condensed chromosomes and were arrested in mitosis (data not shown). Thus, like mos^{xe}, v-*mos* can function to arrest cleavage in developing embryos.

Discussion

We have compared the activities of the v-*mos* and mos^{xe} proteins when expressed in *Xenopus* oocytes and embryos. Microinjection of v-*mos* RNA induced GVBD in oocytes in a dose-dependent manner comparable to mos^{xe}. Oocytes that had been injected with a mos^{xe}-specific antisense oligonucleotide, rendering then insensitive to progesterone treatment, were induced to mature by microinjection of v-*mos* RNA. Immunoprecipitation of the v-*mos* and mos^{xe} proteins expressed in oocytes showed that equivalent amounts of the two proteins were synthesized; however, the v-*mos* protein was much more active in in vitro kinase assays. Similar results were obtained when the two proteins were expressed in mammalian COS-1 cells, demonstrating that the v-*mos* protein possesses much greater kinase activity than the mos^{xe} protein as measured by autophosphorylation in vitro. Like mos^{xe}, v-*mos* was able to induce cleavage arrest of mitotic *Xenopus* embryos. Thus, it would seem likely that both the mos^{xe} and v-*mos* proteins can interact with the same cellular substrates.

We were somewhat surprised to find similar frequencies of GVBD in oocytes injected with either v-*mos* RNA or mos^{xe} since the v-*mos* and mos^{xe} genes transform murine NIH3T3 cells with widely different efficiencies (Freeman et al., 1989). This could be explained if the two proteins have similar substrate affinities in *Xenopus* oocytes and if the mos^{xe} protein has a much lower affinity for murine substrates. The testing of this hypothesis awaits the identification of the substrates for the mos^{xe} and v-*mos* proteins in oocytes and in transformed cells. Alternatively, the enzymatic activity of the v-*mos* protein could be greater than that of the mos^{xe} protein. Our in vitro studies demonstrating that the v-*mos* protein possesses significantly greater kinase activity than the mos^{xe} protein would seem to support this second hypothesis. However, our results from a previous study comparing the kinase and transforming activities of several v-*mos* mutants suggest that the extent of in vitro autophosphorylation may not necessarily reflect the level of in vivo kinase activity of *mos* (Freeman and Donoghue, 1989). Thus, the v-*mos* and *mos*^{xe} proteins may have similar enzymatic activities in *Xenopus* oocytes yet differ greatly in their ability to autophosphorylate in vitro.

Experiments using *mos*^{xe}-specific antisense oligonucleotides have provided insight into the temporal relationship between *mos*^{xe} action and some of the events associated with maturation. Since GVBD induced by either progesterone or insulin is blocked by *mos*^{xe} antisense oligonucleotides (Sagata et al., 1988), *mos*^{xe} must function downstream of the point where the pathways for progesterone and insulin-induced maturation merge. This is further substantiated by the demonstration that microinjection of *mos*^{xe} antisense oligonucleotides inhibits maturation induced by the p21^{ras} protein (Barrett et al., 1990). The endogenous *Xenopus* c-*ras* protein is believed to mediate insulin-induced maturation but not maturation in response to progesterone (Deshpande et al., 1987; Korn et al., 1987). Two kinase activities normally associated with maturation, histone H1 kinase activity, and ribosomal protein S6 kinase activity, are abolished by injection of oocytes with *mos*^{xe} antisense oligonucleotides (Barrett et al., 1990). Histone H1 kinase activity has been associated with the cdc2 protein kinase (Arion et al., 1988), a subunit of active MPF (Dunphy et al., 1988; Gautier et al., 1988). In progesterone-stimulated oocytes, S6 kinase activity peaks at approximately the same time that MPF activation is maximal (Cicirelli et al., 1988). Thus, as might be expected, active MPF cannot be recovered from progesterone-treated oocytes preinjected with *mos*^{xe} antisense oligonucleotides (Sagata et al., 1989*a*). Clearly, the expression of *mos*^{xe} protein is a prerequisite for MPF activation in oocytes.

The induction of GVBD as well as oncogenic transformation by v-*mos* and *mos*^{xe} is likely to be a function of *mos* serine/threonine kinase activity. This is supported by the observation that a point mutation in the ATP-binding domain of the *mos*^{xe} protein abolishes the ability of *mos*^{xe} to transform cells or induce GVBD in oocytes (Hannink and Donoghue, 1985; Freeman et al., 1989). Although we do not detect in vitro kinase activity associated with the *mos*^{xe} protein immunoprecipitated from microinjected oocytes or transfected COS-1 cells, in vitro phosphorylation of *mos*^{xe} has been reported in kinase assays performed on immunoprecipitates from progesterone-matured oocytes (Watanabe et al., 1989). The apparent discrepancy between these two results may be due to a number of factors. As mentioned above, our kinase assays were performed on *mos*^{xe} protein immunoprecipitated from a small number of microinjected oocytes, whereas the kinase assay of Watanabe et al. (1989) utilized endogenous *mos*^{xe} protein immunoprecipitated from as many as 1,000 progesterone-matured oocytes. In addition, different immunological reagents were used to immunoprecipitate the *mos*^{xe} protein. For example, the COOH-terminal antipeptide serum used in our experiments was raised against a peptide that is a subset of the epitope recognized by the antibody of Watanabe et al. (1989); thus, these antisera are similar, although not identical. The other antibody which we used, the anti(37–55)-serum, recognizes a region conserved in both the v-*mos* and *mos*^{xe} proteins; this is the only *mos*-specific antibody known that permits v-*mos* autophosphorylation in immune complex kinase assays (Maxwell and Arlinghaus, 1986*b*; Singh et al., 1986*a*). Obviously, a direct comparison of the kinase activities of v-*mos* and *mos*^{xe}, using the conditions and reagents described by Watanabe et al. (1989) may be needed to resolve this issue. Nonetheless, the results reported here clearly indicate a significant difference in the in vitro kinase activity of the v-*mos* and *mos*^{xe} proteins, despite their similar activity in the microinjection experiments described above.

Since the events initiated by *mos* are most likely a function of phosphorylation, what is it that *mos* phosphorylates? As described above, *mos* functions as an activator of MPF in oocytes. MPF exists in a latent form in oocytes and appears to be activated, at least in part, by phosphorylation (Gerhart et al., 1984; Cyert and Kirschner, 1988). In several species, activated MPF appears to consist of a complex containing the cdc2 protein kinase and a cyclin protein (Draetta et al., 1989; Pines and Hunter, 1989; Labbe et al., 1989*a*). Cyclins comprise a family of homologous proteins that were originally identified by their dramatic accumulation during interphase and subsequent destruction at the metaphase–anaphase transition (Evans et al., 1983). The phosphorylation state of the cdc2 protein appears to regulate MPF kinase activity. Although it has been suggested that the most highly phosphorylated forms of the cdc2 protein associate with cyclin during G2 phase (Draetta and Beach, 1988; Pines and Hunter, 1989), tyrosine dephosphorylation of the cdc2 protein during mitosis correlates with maximum histone H1 kinase activity and is thought to be a critical event in fully activating MPF (Labbe et al., 1989*b*; Dunphy and Newport, 1989; Morla et al., 1989). The kinases that phosphorylate the cdc2 protein in vivo and the mechanism by which it is dephosphorylated remain to be identified; although in fission yeast, both protein kinases (Russell and Nurse, 1987*a*,*b*) and protein phosphatases (Ohkura et al., 1989; Booher and Beach, 1989) which may regulate cdc2 activity have been isolated. Cyclin in also phosphorylated in vivo and can be phosphorylated by the cdc2 protein kinase in vitro (Pines and Hunter, 1989; Meijer et al., 1989). Moreover, the phosphorylation of cyclin has recently been shown to correlate with histone H1 kinase activation in sea urchin eggs (Meijer et al., 1989). Thus, both the cdc2 protein and the cyclin protein are potential substrates for the *mos* protein kinase.

The rapid and complete inactivation of MPF at the metaphase–anaphase transition is likely to be, at least in part, the result of proteolytic degradation of cyclin (Murray et al., 1989). The finding that *mos*^{xe} degradation closely parallels MPF inactivation in fertilized *Xenopus* eggs (Watanabe et al., 1989), raises the possibility that *mos* may act to inhibit cyclin proteolysis. This possibility is further suggested by the association of *mos*^{xe} with CSF, an activity that can stabilize MPF activity in cleaving *Xenopus* embryos (Sagata et al., 1989*b*). This could occur directly, if phosphorylation of cyclin by *mos* rendered cyclin less sensitive to proteolysis, or indirectly, if *mos* stabilized cyclin by phosphorylating and inhibiting a protease. Thus, two possible mechanisms for a role for *mos* in a stabilizing MPF involve (*a*) regulation of the phosphorylation state of the cdc2 protein, or (*b*) the inhibition of cyclin degradation.

The ability of *mos* to function as an activator of MPF suggests an attractive model for oncogenic transformation of somatic cells by v-*mos*. The transformed phenotype may be the consequence of inappropriate *mos*-induced cell cycle transitions. Our demonstration that v-*mos* induces GVBD in oocytes independent of *mos*^{xe} translation is important because it demonstrates that v-*mos* can directly interact with components of the cell cycle machinery. However, the observation that v-*mos* can block the cell cycle by inducing mitotic arrest in *Xenopus* embryos suggests that *mos* may also function after the initiation of mitosis. It is possible that elevated levels of *mos* expression in somatic cells may also result in mitotic arrest. Interestingly, the time at which v-*mos* synthesis peaks in cells acutely infected with Moloney murine sarcoma virus immediately precedes a wave of cell death (Papkoff et al., 1982). Thus, low levels of *mos* protein may be involved in inducing mitosis, whereas higher levels may act to sustain the mitotic state, possibly leading to cell death. Consequently, transformed cells may express a level of *mos* protein that is insufficient to induce mitotic arrest but that is capable of activating MPF. Our results, demonstrating that v-*mos* can directly influence the *Xenopus* cell cycle in inducing GVBD and arresting mitotic cleavage, strongly support the idea that v-*mos* interacts closely with the cell cycle machinery in *mos*-transformed cells.

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