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 can induce the maturation of prophase-arrested Xenopus oocytes. Recently, we showed that mos 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 protein in stimulating Xenopus oocytes to complete meiosis. Microinjection of in vitro synthesized RNAs encoding either the mos or v-mos proteins stimulates resting oocytes to undergo germinal vesicle breakdown. Microinjection of an antisense oligonucleotide spanning the initiation codon of the mos gene blocked progesterone-induced oocyte maturation. When oocytes were microinjected first with the mos 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 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 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, 1985a; 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) is required for the maturation of Xenopus oocytes (Sagata et al., 1988). Microinjection of oocytes with mos-specific antisense oligonucleotides prevents hormone-induced germinal vesicle breakdown (GVBD). Moreover, prophase-arrested oocytes can be induced to undergo GVBD by microinjection of in vitro transcribed mos RNA, demonstrating that expression of the mos protein is sufficient for reinitiation of normal meiosis (Freeman et al., 1989; Sagata et al., 1989a). mos RNA is present during oocyte growth and maturation and persists in the developing embryo through blastulation; however, the mos 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-specific antisense oligonucleotides inhibited GVBD induced by injection of the p21 protein (Barrett et al., 1990). Hormone-induced maturation of oocytes is known to stimulate two kinase activities: one which phosphorylates

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 antisense oligonucleotides (Barrett et al., 1990). Histone H1 kinase activity has recently been attributed to the cdc2 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 is involved in the activation or stabilization of MPF.

A second function for the mos protein has been suggested based on experiments in which mos RNA was microinjected into cleaving Xenopus embryos (Sagata et al., 1989b). 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 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 protein from egg extracts with mos antisense oligonucleotides (Barrett et al., 1990). Histone H1 kinase activity demonstrated by in vitro autophosphorylation by the mos protein possesses an intrinsic protein kinase 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. In addition to its transforming activity, the v-mos protein possesses an intrinsic protein kinase activity demonstrated by in vitro autophosphorylation (Maxwell and Arlinghaus, 1985a). 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 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. Induction of GVBD by v-mos was not affected by preinjection of the oocytes with mos-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 protein. Finally, we show that v-mos can function to arrest cleaving Xenopus embryos and thus can substitute for the mos protein as a component of CSF.

**Materials and Methods**

**In Vitro Transcription of RNA**

The wild type mos/pSP64(polyA) plasmid and the mutant mos (RgO) 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 Biotech, Madison, WI) upstream of the synthetic poly(A) tract. The v-mos, mos, and mos (RgO) plasmids were then linearized at a unique restriction enzyme site downstream of the poly(A) tract and used as templates for transcription of capped and polyadenylated RNAs by SP6 RNA polymerase (Promega Biotech) 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 [35S]methionine.

**Microinjection of RNA into Xenopus Oocytes and Embryos**

Stage VI oocytes were manually dissected from ovaries surgically removed from female Xenopus (Xenopus l. 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(NO3)2, 0.41 mM CaCl2, 0.82 mM MgSO4, 2.4 mM NaHCO3, 10 mM Hepes pH 7.4, 0.1 mM 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 10 U of pregnant mare serum gonadotropin (Calbiochem-Behring Corp., La Jolla, CA) 3–10 h 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 MgSO4, 2 mM CaCl2, 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 ng/ml) just before completion of the first cleavage.

**Microinjection of Antisense Oligonucleotides**

An antisense oligonucleotide that spans the mos start codon and is complementary to the nucleotide sequence (−)18 to 7 of the mos 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 ng/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 ng/ml). Oocytes were scored for GVBD as described above.

**Immunoprecipitation and In Vitro Kinase Assay of mos and v-mos Expressed in Oocytes**

Microinjected oocytes were labeled by incubation in MBS-H containing 0.5 mCi/ml each of [35S]cysteine and [35S]methionine for 12 h. Oocytes were rinsed twice in MBS-H and lysed in 2–3 μl per oocyte of Triton NP-40 buffer (10 mM Tris HCl, pH 6.8, 5 mM EDTA, 150 mM NaCl, 1% NP-40, 10 μg/ml aprotinin) 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 [35S]-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 antisend-55)-serum (Gallick et al., 1985), which recognizes the NH2-terminus of both v-mos and mos, or an antisend-55)-serum corresponding to COOH-terminal 12 amino acids of mos 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 S. aureus bacteria (Staphylococcus aureus bacterium, 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 Triton 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 et al., 1988).
Expression of mos\(^{\text{ex}}\) and v-mos in COS-1 Cells

The v-mos and mos\(^{\text{ex}}\) 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 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 \text{mCi/ml} each of \(\text{[35S]cysteine}\) and \(\text{[35S]methionine}\) in DME lacking cysteine and methionine. Cells were lysed in NP-40 buffer and immunoprecipitated with either anti (\(\text{[35S]}\)-) serum. For in vitro kinase assays, COS-1 cells were transfected with the v-mos and mos\(^{\text{ex}}\) genes as described above and lysed in Tris/NP-40 buffer containing 2 mM DTT. Cell lysates were immunoprecipitated and subjected to in vitro kinase assays as described previously (Maxwell and Arlinghaus, 1985; Singh et al., 1988). The phosphorylated proteins were analyzed by 15% SDS-PAGE and autoradiography.

Expression of mos\(^{\text{ex}}\) and v-mos in COS-1 Cells

The v-mos and mos\(^{\text{ex}}\) 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 linkers, 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 \text{mCi/ml} each of \(\text{[35S]cysteine}\) and \(\text{[35S]methionine}\) in DME lacking cysteine and methionine. Cells were lysed in RIPA buffer and immunoprecipitated with either anti (37-55)-serum or anti(mos\(^{\text{ex}}\))-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\(^{\text{ex}}\) genes as described above and lysed in Tris/NP-40 buffer containing 2 mM DTT. Cell lysates were immunoprecipitated and subjected to in vitro kinase assays as described previously (Maxwell and Arlinghaus, 1985; 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\(^{\text{ex}}\) RNA into phase-arrested Xenopus oocytes induces oocyte maturation, characterized by GVBD, in a dose-dependent manner (Freeman et al., 1989; Sagata et al., 1989a). 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\(^{\text{ex}}\) 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\(^{\text{ex}}\) RNA. At the lowest and highest concentrations tested, both v-mos and mos\(^{\text{ex}}\) RNAs induced GVBD with roughly comparable efficiencies; however, at intermediate concentrations v-mos RNA was 4-6-fold more effective than mos\(^{\text{ex}}\) 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\(^{\text{ex}}\) RNA. Although the amount of mos\(^{\text{ex}}\) 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\(^{\text{ex}}\) in this capacity.

Antisense Inhibition of mos\(^{\text{ex}}\) 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\(^{\text{ex}}\) 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\(^{\text{ex}}\) antisense oligonucleotides, which can inhibit hormone-induced maturation of Xenopus oocytes by preventing mos\(^{\text{ex}}\) translation (Sagata et al., 1988), and subsequently microinjected v-mos RNA in an effort to overcome the inhibition of translation of mos\(^{\text{ex}}\). For the first microinjection, oocytes were injected with an antisense oligonucleotide that spans the initiator methionine codon for mos\(^{\text{ex}}\). 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 [\(\text{[35S]}\)]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-
oocytes were also [35S]methionine labeled and immunoprecipitated with anti(37-55)-serum for comparison (lane 1). Immunoprecipitation of the 41-kDa mos" protein and 39-kDa 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")-serum did not immunoprecipitate any v-mos protein (lane 9).

Approximately 16 times more mos" 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" 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" in microinjected oocytes. Oocytes were microinjected with 50 nl of 2 mg/ml of v-mos or mos" RNA as described in Materials and Methods. After the injections, oocytes were cultured for 12 h in MBS-H containing 500 #Ci/ml each of [35S]cysteine and [3S]methionine. Progesterone-treated oocytes were cultured as above except that the media also contained 15 #M 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"-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" proteins, shown in lanes 1, 2, and 5-8; or with anti(mos")-serum, lanes 3, 4, 9, and 10. The arrows indicate the positions of the mos" (upper arrow) and the v-mos proteins (lower arrow). Molecular weight markers are given on the right side of the fluorograph.
the mos protein possesses very little, if any, in vitro kinase activity.

To examine the difference in kinase activity between the v-mos and mos 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 [35S]methionine-labeled lysates prepared from v-mos- or mos-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 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 RNA injected oocytes described above. Thus, the v-mos and mos proteins differ markedly in their ability to be phosphorylated in vitro yet remarkably in their ability to be phosphorylated in vitro yet

Figure 4. In vitro kinase assay of v-mos and mos expressed in oocytes. Oocytes were microinjected with v-mos and mos 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-[32P]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-injected oocytes and lanes 5-8 are from v-mos-injected oocytes. Immunoprecipitations were performed with anti(mos)-serum, shown in lanes 1, 2, 7, and 8; or with anti(37-55)-serum, which recognizes the NH2-termini of both v-mos and mos proteins, 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 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 protein (Fig. 3, lanes 5 and 7), phosphorylation of the v-mos protein was ~10 times that of the background level in the corresponding region of the mos 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,

Figure 5 (A) Transient expression of v-mos and mos in COS-1 cells. The v-mos and the mos 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 [35S]cysteine and [35S]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 transfection; (lane 3), v-mos transfection. The arrows indicate the positions of the mos (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 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 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.
they exert similar biological activities when expressed in oocytes.

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

Recently, the mos ~ 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., 1989b). 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 ~ 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 ~, is able to induce mitotic arrest in Xenopus embryos.

To test whether v-mos can function in place of mos ~ 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 ~ RNA, v-mos RNA, or RNA synthesized from the mos ~ gene (Freeman et al., 1989) which encodes a point mutation in the canonical ATP-binding site. Microinjection of 30 ng of mos ~ 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 ~ RNA (Fig. 6). However, microinjection of mos ~ 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 ~, v-mos can function to arrest cleavage in developing embryos.

**Discussion**

We have compared the activities of the v-mos and mos ~ 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 ~. Oocytes that had been injected with a mos ~-specific antisense oligonucleotide, rendering them insensitive to progesterone treatment, were induced to mature by microinjection of v-mos RNA. Immunoprecipitation of the v-mos and mos ~ 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 ~ protein as measured by autophosphorylation in vitro. Like mos ~, v-mos was able to induce cleavage arrest of mitotic Xenopus embryos. Thus, it would seem likely that both the mos ~ 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 ~ since the v-mos and mos ~ 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 ~ protein has a much lower affinity for murine substrates. The testing of this hypothesis awaits the identification of the substrates for the mos ~ 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 ~ protein. Our in vitro studies demonstrating that the v-mos protein possesses significantly greater kinase activity than the mos ~ 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.
activity of \textit{mos} (Freeman and Donoghue, 1989). Thus, the \textit{v-mos} and \textit{mos}\textsuperscript{ex} proteins may have similar enzymatic activities in \textit{Xenopus} oocytes yet differ greatly in their ability to autophosphorylate in vitro.

Experiments using \textit{mos}\textsuperscript{ex}-specific antisense oligonucleotides have provided insight into the temporal relationship between \textit{mos}\textsuperscript{ex} action and some of the events associated with maturation. Since GVBD induced by either progesterone or insulin is blocked by \textit{mos}\textsuperscript{ex} antisense oligonucleotides (Sagata et al., 1988), \textit{mos}\textsuperscript{ex} 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 \textit{mos}\textsuperscript{ex} antisense oligonucleotides inhibits maturation induced by the p21\textsuperscript{ex} protein (Barrett et al., 1990). The endogenous \textit{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 \textit{mos}\textsuperscript{ex} 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 (Duphhy et al., 1988; Gauthier 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 \textit{mos}\textsuperscript{ex} antisense oligonucleotides (Sagata et al., 1989a). Clearly, the expression of \textit{mos}\textsuperscript{ex} protein is a prerequisite for MPF activation in oocytes.

The induction of GVBD as well as oncogenic transformation by \textit{v-mos} and \textit{mos}\textsuperscript{ex} is likely to be a function of \textit{mos} serine/threonine kinase activity. This is supported by the observation that a point mutation in the ATP-binding domain of the \textit{mos}\textsuperscript{ex} protein abolishes the ability of \textit{mos}\textsuperscript{ex} 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 \textit{mos}\textsuperscript{ex} protein immunoprecipitated from microinjected oocytes or transfected COS-1 cells, in vitro phosphorylation of \textit{mos}\textsuperscript{ex} 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 \textit{mos}\textsuperscript{ex} protein immunoprecipitated from a small number of microinjected oocytes, whereas the kinase assay of Watanabe et al. (1989) utilized endogenous \textit{mos}\textsuperscript{ex} protein immunoprecipitated from as many as 1,000 progesterone-matured oocytes. In addition, different immunological reagents were used to immunoprecipitate the \textit{mos}\textsuperscript{ex} 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 \textit{v-mos} and \textit{mos}\textsuperscript{ex} proteins; this is the only \textit{mos}-specific antibody known that permits \textit{v-mos} autophosphorylation in immune complex kinase assays (Maxwell and Arlinghaus, 1986; Singh et al., 1986a). Obviously, a direct comparison of the kinase activities of \textit{v-mos} and \textit{mos}\textsuperscript{ex}, 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 \textit{v-mos} and \textit{mos}\textsuperscript{ex} proteins, despite their similar activity in the microinjection experiments described above.

Since the events initiated by \textit{mos} are most likely a function of phosphorylation, what is it that \textit{mos} phosphorylates? As described above, \textit{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., 1989a). 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, 1989; 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., 1989b; Duphhy 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, 1987a,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 \textit{mos}\textsuperscript{ex} 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 \textit{mos}\textsuperscript{ex} degradation closely parallels MPF inactivation in fertilized \textit{Xenopus} eggs (Watanabe et al., 1989), raises the possibility that \textit{mos} may act to inhibit cyclin protein synthesis. This possibility is further suggested by the association of \textit{mos}\textsuperscript{ex} with CSF, an activity that can stabilize MPF activity in cleaving \textit{Xenopus} embryos (Sagata et al., 1989b). This could occur directly, if phosphorylation of cyclin by \textit{mos} rendered cyclin less sensitive to proteolysis, or indirectly, if \textit{mos} stabilized cyclin by phosphorylating and inhibiting a protease. Thus, two possible mechanisms for a role for \textit{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 \textit{mos} to function as an activator of MPF suggests an attractive model for oncogenic transformation of somatic cells by \textit{v-mos}. The transformed phenotype may be the consequence of inappropriate \textit{mos}-induced cell cycle transitions. Our demonstration that \textit{v-mos} induces GVBD in oo-
cytes independent of mos transcription 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.

The authors wish to thank Ralph Arlinghaus for generously providing anti-mos (37–55) antiserum.

This work was supported by grant CA 34456 from the National Institutes of Health (NIH). In addition, generous support from an American Cancer Society Faculty Research Award and from the Markey Foundation is gratefully acknowledged. R. S. Freeman was supported by predoctoral training grant CA 08552 and K. M. Pickham was supported by predoctoral training grant GM 07313 from the NIH.

Received for publication 14 February 1990 and in revised form 13 April 1990.

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