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Xenopus homolog of the *mos* protooncogene transforms mammalian fibroblasts and induces maturation of *Xenopus* oocytes

(protein kinase/meiosis/germinal vesicle breakdown)

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ABSTRACT **The oncogene v-*mos* transforms mammalian fibroblasts and encodes a serine/threonine protein kinase. Expression of the c-*mos* protooncogene is most abundant in germ cells, suggesting a normal role for c-*mos* in meiosis. Here we describe the isolation of cDNA clones containing the complete coding region of the *Xenopus laevis* homolog of c-*mos* (*mos*^{xe}). The *mos*^{xe} gene is transforming when introduced into murine NIH 3T3 cells, and transformation is abrogated by a lysine-to-arginine mutation in the canonical ATP-binding site. Microinjection of *in vitro* transcribed *mos*^{xe} RNA into prophase-arrested *Xenopus* oocytes causes a resumption of meiosis, leading to germinal vesicle breakdown and oocyte maturation. Oocyte maturation was not observed after microinjection of *in vitro* transcribed *mos*^{xe} RNA encoding the lysine-to-arginine mutation. These results demonstrate that the *mos*^{xe}-encoded protein can induce progression through the cell cycle for both meiotic and mitotic cells and that this property is dependent on the presumptive ATP-binding domain in the protein kinase.**

The v-*mos* oncogene, the transforming gene of Moloney murine sarcoma virus, encodes a 37-kDa serine/threonine protein kinase (1, 2). Genomic DNA clones for the cellular homolog of v-*mos* have been isolated from various mammalian species and also from chicken (3–7). All of the c-*mos* genes can transform mouse fibroblasts *in vitro* (4, 5, 7–9), suggesting a conserved biochemical function. Transformation by v-*mos* is abolished by a site-directed mutation in the canonical ATP-binding domain of the v-*mos*-encoded protein, suggesting that transformation is likely to be a function of its intrinsic protein kinase activity (10).

In all species examined, expression of c-*mos* RNA is greatest in adult gonadal tissues (5, 7, 11). In mice, *mos* transcripts are localized in the germ cells and their expression is developmentally regulated (12–14). Recently, the presence of endogenous *mos*-encoded protein was demonstrated in mouse testes (15). These observations suggest an important role for *mos* in mammalian germ cell maturation.

Since the amphibian *Xenopus laevis* (16) provides a convenient system for studying the biochemistry of germ cell development, we have isolated cDNA clones containing the coding region of the *Xenopus* homolog of *mos* (*mos*^{xe}).¶ The *mos*^{xe} gene is transforming when assayed in mouse fibroblasts and transformation is prevented by site-directed mutation of the lysine residue in the predicted ATP-binding domain. Microinjection of *mos*^{xe} RNA induces germinal vesicle breakdown (GVBD) and oocyte maturation and these activities are dependent on an intact protein kinase catalytic domain. These observations provide direct evidence of a function for *mos* in normal germ cell development.

MATERIALS AND METHODS

Isolation of *mos*^{xe} cDNA Clones and Site-Directed Mutagenesis. Nitrocellulose filters prepared from a *Xenopus* oocyte λgt10 cDNA library (17) were screened at low stringency (18) using a ^{32}P-labeled v-*mos* probe. Overlapping deletions of *mos*^{xe} cDNA were cloned into pBluescript KS(−) (Stratagene) and dideoxynucleotide sequencing was performed with Sequenase (United States Biochemical) and adenosine 5′-[α-[^{35}S]thio]triphosphate. The synthetic oligodeoxynucleotide CGGTGGCGCTGCGCAAGGTAAAACGC was used for site-directed mutagenesis (19) of *mos*^{xe} cDNA to replace the codon for lysine-90 with a codon for arginine.

RNA Isolation and Northern Blot Analysis of *mos*^{xe} RNA from Staged Oocytes and Unfertilized Eggs. Unfertilized eggs were obtained as described (20). Oocytes were obtained from collagenase-treated ovaries (5 mg/ml) prior to staging (21) and RNA extraction. Total RNA from oocytes or eggs was fractionated on a 1% agarose/2.2 M formaldehyde gel and transferred to a Nytran membrane (Schleicher & Schuell). Hybridization was performed with a ^{32}P-labeled *mos*^{xe} probe.

Focus Assays and Virus Titers. The wild-type and the mutant *mos*$^{xe(R90)}$ genes were inserted into a Moloney murine leukemia virus (M-MuLV)-derived vector, pDD102 (22), and assayed for focus-forming activity by cotransfection with a DNA clone of replication competent M-MuLV onto NIH 3T3 cells (22). The conditioned medium was also assayed for infectious transforming virus.

***In Vitro* Transcription and Translation of *mos*^{xe} and *mos*$^{xe(R90)}$.** The 5′-capped and polyadenylylated RNAs were transcribed *in vitro* as described (23) from the *mos*^{xe} and *mos*$^{xe(R90)}$ genes cloned into pSP64(poly A) (Promega). RNAs were translated *in vitro* in rabbit reticulocyte lysates (Amersham) containing 50 μCi of [^{35}S]methionine (1000 Ci/mmol; 1 Ci = 37 GBq).

Oocyte Microinjections and Immunoprecipitations. Stage VI oocytes were either treated with progesterone (30 μM) or injected with *in vitro* synthesized RNA (1–100 ng in a vol of 50 nl), and subsequently labeled for ≈16 hr at 18°C with 0.5 mCi of [^{35}S]methionine per ml. Immunoprecipitates of oocyte extracts with anti-*mos*-(37–55) antiserum (2) were collected with *Staphylococcus aureus* bacteria and analyzed by 15% SDS/PAGE and fluorography.

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

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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M25366).

RESULTS AND DISCUSSION

Isolation of cDNA Clones for the *Xenopus* Homolog of the *mos* Protooncogene. We screened a *X. laevis* cDNA library (17) for cellular *mos* sequences and identified six positive clones (Fig. 1). These fell into two classes based on restriction site polymorphisms reflected in part by the presence or absence of a *Bgl* II site. DNA sequencing revealed that these clones contain an open reading frame of 1080 bp, which would encode a 359-amino acid polypeptide (predicted mass of 39.1 kDa). The predicted amino acid sequence of the mos^{xe}-encoded protein shares between 50% and 55% sequence identity with other *mos* proteins (Fig. 2). All of the domains characteristic of protein kinases are highly conserved.

Expression of mos^{xe} RNA During Oocyte Growth and Maturation. Very low levels of *mos* transcripts have been detected in a variety of mouse, monkey, and chicken tissues (5, 7, 11). However, the highest levels of *mos* RNA are present in male and female germ cells (12–14). In mice, *mos* expression first appears during the early stages of oocyte growth and accumulates prior to the second meiotic division. Fertilized eggs, which have completed meiosis, retain a lower level of maternal *mos* transcripts; however, two-cell embryos contain no detectable *mos* RNA (25, 26).

Similar to the pattern of *mos* expression in mammalian oocytes and eggs, mos^{xe} RNA is expressed during maturation of *Xenopus* oocytes (Fig. 3). RNA isolated from oocytes in stages II–VI of development and from unfertilized eggs was subjected to Northern blot analysis. Low levels of a 3.1-kb transcript were detected in stage II oocytes. *mos* RNA accumulated by stage III and similar high levels persisted through stage VI and in unfertilized eggs.

Growing *Xenopus* oocytes are arrested in prophase of the first meiotic division. Steroid hormones, such as progesterone, stimulate oocytes to undergo maturation, characterized by nuclear envelope breakdown, chromosome condensation, and spindle formation, leading to the first meiotic division and polar body extrusion (16). The expression of mos^{xe} in *Xenopus* oocytes, first apparent during prophase I in the growing oocyte and continuing throughout meiosis and in the unfertilized egg, parallels the expression of *mos* in mammalian oocytes.

The mos^{xe} Gene Is Transforming and This Activity Is Abolished by Mutation of a Conserved Lysine Predicted to Be Involved in ATP Binding. The v-*mos* gene, as well as the *mos* protooncogenes from mouse, rat, and chicken, transforms mouse fibroblasts when activated by a retroviral long terminal repeat (LTR) (4, 7, 9). Human and monkey c-*mos* genes are only ≈1% as efficient as v-*mos* in transforming fibroblasts (5, 8). We assayed the mos^{xe} gene for its ability to transform mouse NIH 3T3 cells (Table 1). When expressed under the control of a retroviral LTR, the mos^{xe} gene induces foci in NIH 3T3 cells, although with approximately 1–2% the transforming efficiency of v-*mos*. Thus, the mos^{xe} cDNA is biologically active as assayed by its ability to transform fibroblasts with an efficiency similar to the human and monkey c-*mos* genes.

The conserved lysine residue in the ATP-binding site of all protein kinases is required for the transforming activities of numerous oncogenic protein kinases (27). Substitution of this lysine residue in the v-*mos* protein with arginine results in a stably expressed protein that lacks protein kinase activity and transforming activity (10, 28). To examine the importance of the corresponding lysine in the mos^{xe} protein (lysine-90), we constructed a mutant gene in which this lysine codon was replaced with a codon for arginine. The mutant gene [$mos^{xe(R90)}$] was nontransforming when assayed in NIH 3T3 cells (Table 1). This result reveals that lysine-90 is required for transformation by mos^{xe} and suggests that an ATP-binding site and protein kinase domain are functional components of the mos^{xe} protein.

Characterization of the mos^{xe} Protein. We synthesized RNAs from mos^{xe} cDNA and from the $mos^{xe(R90)}$ mutant gene using SP6 RNA polymerase (23). Rabbit reticulocyte

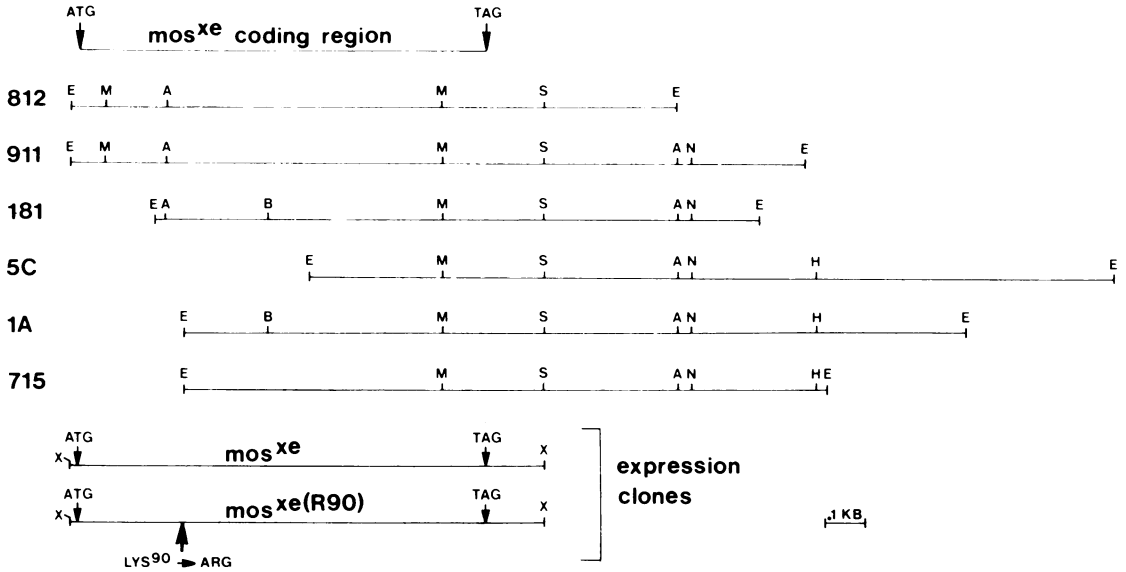

FIG. 1. mos^{xe} cDNA clones obtained from a *Xenopus* oocyte cDNA library. (*Upper*) The mos^{xe} coding region of 1080 base pairs (bp) is shown, encoding a predicted protein of 359 amino acids. The cDNA clones shown represent 2.7 kilobases (kb) of the 3.1-kb mRNA detected in Fig. 3. Thus, these clones lack ≈400 bp of 5′ plus 3′ untranslated sequences including a consensus polyadenylylation signal. Since the *X. laevis* genome has undergone an evolutionary gene duplication event (24), there probably exist two pairs of mos^{xe} genes represented by the restriction site polymorphisms observed in the cDNA clones (e.g., *Bgl* II). The 812 clone has an additional dGMP at nucleotide 723, which is absent from the other clones. (*Lower*) Expression clones used in focus assays and for synthesizing RNA are shown. Expression DNAs were inserted into a retroviral expression vector as an *Xho* I fragment obtained by placing *Xho* I linkers into the *Eco*RI site at the 5′ end of the cDNA and the *Spe* I site in the 3′ untranslated region. The expression DNAs were inserted into pSP64(poly A) for *in vitro* transcription of RNA. The mos^{xe} expression DNAs are derived from clone 812 upstream of the first *Acc* I site and from clone 181 between the first *Acc* I site and the *Spe* I site. R, *Eco*RI; M, *Mst* II; A, *Acc* I; B, *Bgl* II; S, *Spe* I; N, *Nco* I; H, *Hin*dIII; X, *Xho* I.

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XENOPUS MPSPIPVERFLPRDLSPSIDLRPCSSPLELSHRKLPGGLPAC-SGRPRLLPPRLAWCSIDWEQVLLLEPLGSGGFG 75
CHICKEN MPSPIPFNSFLPLELSPSADLRPCSSPVVIPGKDGKAFLGGTPSPRTRRLPPRLAWCSIDWDRLCLLQPLGSGGFG 76
HUMAN   MPSPLALRPYLRSEFSPSVDARPCSSPSELPAKL----LLGATLPRAPRLPRRLAWCSIDWEQVCLLQRLGAGGFG 72
MOUSE   MPSPLSLCRYLPRELSPSVDSRSCSIPLVAPRKAGKLFLGTTP-PRAPGLPRRLAWFSIDWEQVCLMHRLGSGGFG 75

XENOPUS SVYRATYRGETVALKKVKRSTKNSLASRQSFWAELNAARLRHPHVVRVVAASASCPGDPGCPGTIIMEYTGTGTLH 151
CHICKEN AVYKATYHGVTVAVKQVKKSSKNRLASRQSFWAELNVARLQHDNVVRVVAASTCAPASQNSLGTIIMEYVGNVTLH 152
HUMAN   SVYKATYRGVPVAIKQVNKCTKNRLASRRSFWAELNVARLRHDNIVRVVAASTRTPAGSNSLGTIIMEFGGNVTLH 148
MOUSE   SVYKATYHGVPVAIKQVNKCTKDLRASQRSFWAELNIARLRHDNIVRVVAASTRTPEDSNSLGTIIMEFGGNVTLH 151

XENOPUS QRIYG--RSP-PLGAEI----------CMRYARHVADGLRFLHRDGVVHLDLKPANVLLAPGDLCKIGDFGCSQ 212
CHICKEN HVIYGTRDAWRQGEEEEGGCGRKALSMAEAVCYSCDIVTGLAFLHSQGIVHLDLKPANILITEHGACKIGDFGCSQ 228
HUMAN   QVIYGAAGHPEGDAGEPHCRTGGQLSLGKCLKYSLDVVNGLLFLHSQSIVHLDLKPANILISEQDVCKISDFGCSE 224
MOUSE   QVIYGATRSPEPLSC------REQLSLGKCLKYSLDVVNGLLFLHSQSILHLDLKPANILISEQDVCKISDFGCSQ 221

XENOPUS RLREGDEAAGGEPCCTQLRHV---GGTYTHRAPELLKGEPVTAKADIYSFAITLWQMVSRELPYTGDRQCVLYAVV 285
CHICKEN RLEEGLS---------QSHHVCQQGGTYTHRAPELLKGERVTAKADIYSFAITLWQIVMREQPYLGERQYVLYAVV 295
HUMAN   KLEDLLC---------FQTPSYPLGGTYTHRAPELLKGEGVTPKADIYSFAITLWQMTTKQAPYSGERQHILYAVV 291
MOUSE   KLQVLRC---------RQASPHHIGGTYTHQAPEILKGEIATPKADIYSFGITLWQMTTREVPYSGEPQYVQYAVV 288

XENOPUS AYDLRPEMGPLFSHTEE-GRAARTIVQSCWAARPQERPNAEQLLE-RLEQECAMCTGGPPSCSPESNAPPPLGTGL 359
CHICKEN AYNLRPPLAAAIFHESAVGQRLRSIISCCWKADVEERLSAAQLLPSLRA----------------LKENL 349
HUMAN   AYDLRPSLSAAVFEDSLPGQRLGDVIQRCWRPSAAQRPSARLLLVDLTS----------------LKAELG 346
MOUSE   AYNLRPSLAGAVFTASLTGKTLQNIIQSCWEARALQRPGAELLQRDLKA----------------FRGALG 343
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FIG. 2. Alignment of the c-*mos* amino acid sequences (single-letter code) from various species. The predicted amino acid sequence encoded by *mos*^{xe} is aligned with the sequences of the chicken, human, and mouse *mos* proteins (3, 6, 7). Residues conserved between two or more of the *mos* proteins are designated by boldface type. Gaps in the alignment are indicated by dashes.

lysates translate both the wild-type and mutant transcripts into proteins with a relative molecular mass of 41 kDa (Fig. 4*A*). We also found that a polyclonal anti-peptide antiserum (2) could immunoprecipitate the *in vitro* translated *mos*^{xe} and *mos*$^{xe(R90)}$ proteins (see Fig. 4*B*, lane 5).

Xenopus oocytes will efficiently translate *in vitro* transcribed RNAs injected into their cytoplasm (19). *Xenopus* oocytes injected with *mos* RNA translate a 41-kDa protein, which is easily detected by immunoprecipitation with anti-*mos* antiserum and which comigrates with the *in vitro* translated *mos*^{xe} protein (see Fig. 4*B*, lanes 4 and 5).

***mos*^{xe} Induces GVBD in *Xenopus* Oocytes.** Tissue-specific *mos* RNA expression in a variety of organisms suggests a possible role for the *mos* protein in germ cell development. Although *mos*^{xe} transcripts accumulate during oocyte growth, we do not observe detectable levels of *mos*^{xe} protein in fully grown immature oocytes labeled with [^{35}S]methionine and immunoprecipitated with anti-*mos* antiserum (see Fig. 4*B*, lane 1). To determine whether *mos*^{xe} expression affects the maturation of oocytes, we microinjected *in vitro* synthesized and polyadenylylated *mos*^{xe} RNA into stage VI oocytes. In control oocytes, progesterone was used to induce GVBD and maturation, characterized by a well-defined pigmentation change in the animal pole (16). Microinjection of *mos*^{xe} RNA induced GVBD in >85% of injected oocytes (Table 2). The oocytes induced to mature by injection of *mos*^{xe} RNA underwent GVBD later (7 hr postinjection) than oocytes induced with progesterone (4 hr postinjection). We also found that injection of ≥50 ng of RNA was required to produce GVBD in a majority of oocytes, suggesting that a threshold level of *mos*^{xe} is required for oocyte maturation.

To confirm that GVBD resulted directly from the presence of *mos*^{xe} RNA, we injected *in vitro* transcribed *mos*$^{xe(R90)}$ RNA into oocytes. In experiments performed in parallel with wild-type *mos*^{xe} injections, *mos*$^{xe(R90)}$ RNA failed to induce GVBD (Table 2) even when as much as 100 ng was injected. This demonstrates that the induction of GVBD is a direct consequence of the presence of *mos*^{xe} and not an artifact of the microinjections. The inability of the *mos*$^{xe(R90)}$ mutant to induce GVBD indicates a requirement for an intact ATP-binding site and protein kinase activity.

To identify the *mos*^{xe} protein in oocytes induced to mature with *mos*^{xe} RNA, we subjected [^{35}S]methionine-labeled oocytes to immunoprecipitation with anti-*mos* antiserum (Fig. 4*B*). The 41-kDa *mos*^{xe} protein was easily detected in *mos*^{xe}-injected oocytes that had undergone GVBD but was not detected in oocytes injected with <50 ng of *mos*^{xe} RNA (Fig. 4*C*) or in mock-injected control oocytes. Lower amounts of *mos*$^{xe(R90)}$ protein were detected in oocytes injected with the mutant *mos* RNA (Fig. 4*B*, lane 3). Low

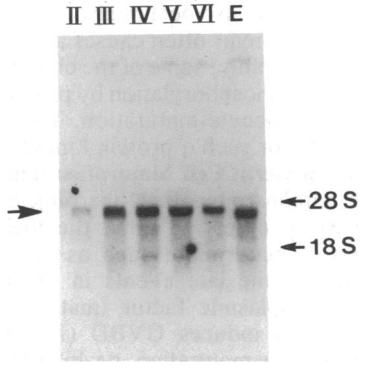

FIG. 3. Northern blot analysis of *mos*^{xe} RNA from staged oocytes and unfertilized eggs. Each lane of the Northern blot represents total RNA from five oocytes (stages II–VI) or unfertilized eggs. Hybridization was performed with a ^{32}P-labeled *mos*^{xe} probe. Lanes labeled II–VI correspond to RNA isolated from oocytes in stages II–VI. Lane E contains RNA from unfertilized eggs. The arrow to the left designates the 3.1-kb *mos*^{xe} transcript. 28S and 18S ribosomal RNAs are indicated.

Table 1. Transforming activities of the *mos*^{xe} and *mos*$^{xe(R90)}$ genes in mouse NIH 3T3 cells

mos DNA	Focus assay, ffu/μg	Virus titer, ffu/ml
mos$^{xe(R90)}$	0	0
mos^{xe}	1.5×10^1	1.8×10^2
v-*mos*	$>1 \times 10^3$	8.5×10^3

Focus assay results are presented as focus-forming units (ffu) per μg of transfected DNA. Virus titers are described in focus-forming units per ml of conditioned medium. Experiments were repeated in triplicate.

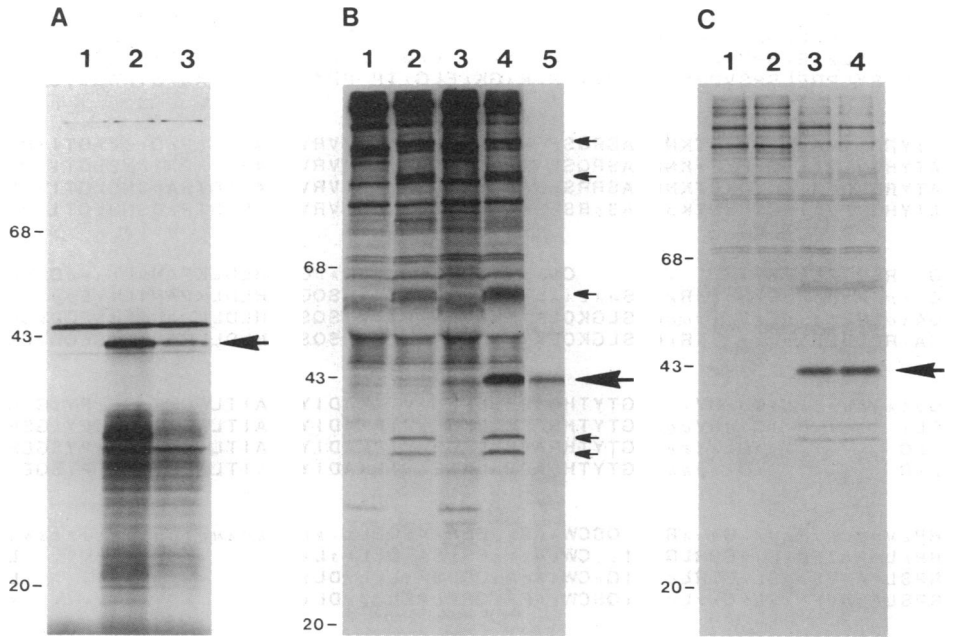

FIG. 4. (*A*) *In vitro* translations of *mos*^{xe} and *mos*$^{xe(R90)}$ RNAs. 5′-Capped and polyadenylylated RNAs were transcribed *in vitro* and translated in rabbit reticulocyte lysates containing [^{35}S]methionine. Lanes: 1, mock translation; 2, *mos*$^{xe(R90)}$ translation; 3, *mos*^{xe} translation. Arrow indicates the mutant and wild-type *mos*^{xe}-encoded proteins. Faster migrating proteins specific to lanes 2 and 3 are likely to be *mos*^{xe} internal initiation products since they are not immunoprecipitated by an N-terminal-derived *mos* antiserum as shown in *B* (lane 5). (*B*) Translation of *mos*^{xe} RNA and immunoprecipitation of *mos*^{xe} protein in microinjected *Xenopus* oocytes. After injection of RNA or addition of progesterone, eight oocytes were labeled for ≈16 hr with [^{35}S]methionine. Immunoprecipitation with anti-*mos*-(37–55) antiserum was performed (2) using a low detergent buffer (20 mM Tris, pH 7.6/100 mM NaCl/1% Triton X-100/1 mM phenylmethylsulfonyl fluoride). Lanes: 1, mock-injected oocytes; 2, progesterone-treated oocytes; 3, *mos*$^{xe(R90)}$-injected oocytes; 4, *mos*^{xe}-injected oocytes; 5, *in vitro* translated *mos*^{xe} protein. Oocytes were injected with 50 ng of RNA. The *mos*^{xe} protein is indicated by the large arrow. Smaller arrows indicate other proteins in lanes 2 and 4 discussed in the text. (*C*) Extracts from oocytes injected with various amounts of *mos*^{xe} RNA and labeled for ≈16 hr with [^{35}S]methionine were immunoprecipitated with anti-*mos*-(37–55) antiserum. Lanes: 1, 1 ng of RNA; 2, 10 ng of RNA; 3, 50 ng of RNA; 4, 100 ng of RNA. All oocytes injected with 50 or 100 ng of *mos*^{xe} RNA underwent GVBD, whereas GVBD did not occur in any oocytes corresponding to lanes 1 and 2. The *mos*^{xe} protein is designated by an arrow. Unlike the samples in *B*, these immune complexes were washed exhaustively in a high detergent (RIPA) buffer (10 mM sodium phosphate, pH 7.0/150 mM NaCl/1% Nonidet P-40/1% sodium deoxycholate/0.1% SDS/1% Trasylol). Samples were analyzed by 15% SDS/PAGE and fluorography. The position of molecular mass markers is indicated in kDa.

levels of the mutant protein could be the result of a block in translation of *mos*$^{xe(R90)}$ RNA or possibly rapid degradation of the mutant RNA or protein. Both the *mos*^{xe} and *mos*$^{xe(R90)}$ proteins can be stably expressed in mammalian COS-1 cells (data not shown). By pulse-labeling injected oocytes, we have found that the *mos*^{xe} protein is synthesized within 2 hr of microinjection of *mos*^{xe} RNA (data not shown). The observations that exogenous *mos*^{xe} is rapidly translated in oocytes and that the start of *mos*^{xe}-induced GVBD is delayed with respect to progesterone induction suggest that *mos*^{xe} activation is an early event in oocyte maturation.

In addition to the *mos*^{xe} protein, several other proteins are visible in immunoprecipitations of [^{35}S]methionine-labeled oocytes, particularly when the immune complexes are prepared with a low detergent buffer (Fig. 4, compare *B* and *C*). Five major differences were observed between immunoprecipitated proteins from uninduced and induced oocytes (see bands designated with small arrows in Fig. 4*B*, lanes 2 and 4). These protein modifications are identical in oocytes induced to mature with either progesterone or injection of *mos*^{xe} RNA. Total protein synthesis is known to increase ≈2-fold shortly after progesterone treatment (29); however, few newly synthesized proteins have been identified. A burst of protein phosphorylation also occurs prior to GVBD (16) and phosphorylation of proteins often causes altered electrophoretic mobility. Conceivably, some of the changes we observe could be the result of phosphorylation by protein kinases that are activated during oocyte maturation. The *mos*^{xe} protein may be a candidate for such a protein kinase.

Table 2. Microinjection of *mos*^{xe} and *mos*$^{xe(R90)}$ into *Xenopus* oocytes

Oocyte treatment	Fraction of oocytes having undergone GVBD
mos^{xe}	7/8 (≥50 ng of RNA)
	1/8 (<50 ng of RNA)
mos$^{xe(R90)}$	0/8
Progesterone	8/8

Stage VI oocytes were either treated with progesterone (30 μM) or injected with *in vitro* synthesized RNA. *mos*$^{xe(R90)}$ RNA (50 ng) was injected into oocytes and either 50–100 ng or <50 ng of *mos*^{xe} RNA was injected as shown. Oocytes were incubated for ≈16 hr at 18°C and then scored for GVBD by the appearance of a white spot in the pigmented animal pole, which was confirmed by manual dissection. Values represent the mean for the fraction of oocytes that had undergone GVBD from several experiments.

Role for *mos*^{xe} in Germ Cell Maturation and Transformation. We have demonstrated that microinjection of *mos*^{xe} RNA into *Xenopus* oocytes can mimic the effects caused by known inducers of maturation, such as progesterone and insulin (16). One of the late events in maturation is the activation of a cytoplasmic factor (maturation-promoting factor, MPF), which induces GVBD (16). MPF activity oscillates during oocyte maturation, peaking at metaphase of both the first and second meiotic divisions (30). However, MPF activity is not limited to meiosis as it apparently induces the mitotic state in all eukaryotic cells (31). Thus, the factor responsible for GVBD in oocytes is similar to that which induces mitosis in somatic cells.

In fission yeast, several mutants that affect cell cycle regulation have been identified. Two of these mitotic regulators (*nim1* and *wee1*) are protein kinase homologs and may

interact via phosphorylation to modulate the activity of *cdc2* (32, 33), the protein kinase component of MPF (34, 35). Both the phosphorylation state and protein kinase activity of *cdc2* have been associated with the events initiated by MPF (36–38). However, the biochemical mechanism of MPF activation is not known and homologs for the fission yeast *nim1* and *wee1* protein kinases have not been identified in other organisms. Our data are consistent with a role for the mos^{xe} protein in oocyte maturation, as either a direct or indirect activator of MPF.

In *Xenopus* oocytes, microinjection of the transforming *ras* protein induces maturation (39, 40). Injection of monoclonal antibodies that inactivate c-*ras* also inhibit maturation induced by insulin but not progesterone (41, 42). This suggests that there are two pathways leading to oocyte maturation: one induced by insulin and mediated by c-*ras*, and the other induced by progesterone. In somatic cells, insulin and many other mitogens interact with cell-surface transmembrane receptors with intrinsic tyrosine protein kinase activity (43). Oncogenic transformation by a variety of tyrosine protein kinases is abrogated by antibodies against the c-*ras* protein, suggesting that c-*ras* participates in this pathway of signal transduction. However, transformation by v-*mos* is not affected by anti-*ras* antibodies, suggesting that *mos* transforms fibroblasts by either a distinct pathway or at a point more proximal to control of the cell cycle (44). It seems likely that the role of the mos^{xe} protein in oncogenic transformation is an extension of its function in oocyte maturation, and that these events occur independently of transmembrane signal transduction pathways.

While this work was nearing completion, Sagata *et al.* (45) described incomplete cDNA clones and a genomic clone of the mos^{xe} gene. When their sequence is compared with that reported here, the two sequences of the predicted mos^{xe} protein are very similar, differing by only two amino acid changes. Our results, obtained by significantly different experimental approaches, provide direct evidence for the induction of oocyte maturation by the mos^{xe} protein kinase.

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