

Mechanistic Studies of the Mitotic Activation of Mos

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The protein kinase Mos is responsible for the activation of MEK1 and p42 mitogen-activated protein kinase during *Xenopus* oocyte maturation and during mitosis in *Xenopus* egg extracts. Here we show that the activation of Mos depends upon the phosphorylation of Ser 3, a residue previously implicated in the regulation of Mos stability; the dephosphorylation of Ser 105, a previously unidentified phosphorylation site conserved in Mos proteins; and the regulated dissociation of Mos from CK2 β . Mutation of Ser 3 to alanine and/or mutation of Ser 105 to glutamate produces a Mos protein that is defective for M-phase activation, as assessed by in vitro kinase assays, and defective for induction of oocyte maturation and maintenance of the spindle assembly checkpoint in extracts. Interestingly, Ser 105 is situated at the beginning of helix α C in the N-terminal lobe of the Mos kinase domain. Changes in the orientation of this helix have been previously implicated in the activation of Cdk2 and Src family tyrosine kinases. Our work suggests that Ser 105 dephosphorylation represents a novel mechanism for reorienting helix α C.

The Mos oncoprotein is a mitogen-activated protein kinase (MAPK) kinase that functions in oocyte maturation in fish, frogs, and mammals (8, 18, 20, 35, 36, 40). In immature *Xenopus* oocytes, the Mos message is present but is translated slowly (35), and as a consequence the Mos protein is present at very low levels. In response to the maturation-inducing hormone progesterone, the translation of Mos increases (35). This is thought to be due to the phosphorylation of the translational regulators CPEB and Maskin by Eg2/Aurora A (23, 30) and the stabilization of the Mos protein through the phosphorylation of Ser 3 (27, 28, 37). Cdk1/Cdc2 (4), p42 MAPK (22), and Mos itself (27) have all been proposed as Ser 3 kinases, although others have argued that none of these protein kinases is responsible (37). The progesterone-induced increases in Mos translation and stability cause Mos levels to rise, which leads to the activation of MEK1, p42 MAPK, and Rsk1/2. These protein kinases reinforce the progesterone-induced activation of Cdk1 through positive feedback loops and help establish the metaphase II arrest state of the mature oocyte (12, 26).

After fertilization, Cdk1 is inactivated as a result of cyclin degradation. This is followed by Mos degradation and the inactivation of MEK1 and p42 MAPK (42). The inactivation of p42 MAPK accompanies the completion of meiosis II and is required for the subsequent initiation of the first mitotic M phase (1, 3, 41). A small proportion of the p42 MAPK then becomes transiently activated during mitosis (14, 44).

Most of the work to date on Mos regulation has focused on Mos translation and stability. However, there have been some indications of additional levels of regulation as well. For example, Chen and Cooper presented evidence that the phosphorylation of Ser 3 promotes the interaction of Mos with MEK1 and promotes the activation of MEK1 by Mos (5). This suggests that Mos is regulated not only at the level of Mos

abundance but also at the level of Mos activity. However, others reported that the Mos-S3A mutant was indistinguishable from wild-type Mos in terms of its ability to induce maturation in oocytes and cytosolic factor (CSF) arrest in cleaving embryos, raising questions about the functional significance of Ser 3 phosphorylation (13). Chen and coworkers also showed that the regulatory subunit of CK2, CK2 β , serves as a negative regulator of Mos (6, 7). Recently, Lieberman and Ruderman corroborated these findings and demonstrated that amino acids 52 to 115 of Mos constitute a CK2 β -interacting surface (21). These studies left open the question of whether the inhibition of Mos by CK2 β was constitutive or regulated.

Recently we showed that Mos was required for the Cdk1-dependent activation of p42 MAPK in *Xenopus* egg extracts (44). Immunodepleting Mos from cycling extracts eliminated the transient activation of p42 MAPK that normally occurs during mitosis, and depleting it from cycloheximide-treated interphase extracts prevented nondestructible cyclin from bringing about p42 MAPK activation (44). Given that there is no Mos synthesis in cycloheximide-treated extracts, this indicated that cyclin must cause Mos to be converted from an inactive form to an active form.

Here we have identified three mechanisms that contribute to the mitotic activation of Mos: the regulated dissociation of CK2 β from Mos, the phosphorylation of Ser 3, and the dephosphorylation of Ser 105. Ser 105 lies at the beginning of the conserved helix α C, whose positioning is critical in the regulation of cyclin-dependent kinase 2 and Src family kinases. We conjecture that the dephosphorylation of Ser 105 represents a novel mechanism for conditionally orienting this helix.

MATERIALS AND METHODS

Preparation and manipulation of egg extracts. Demembrated frog sperm nuclei, cycloheximide-treated interphase egg extracts, CSF egg extracts, and cycling egg extracts were prepared as previously described (25, 39). To drive interphase extracts into a permanent mitotic state, extracts were incubated with 200 nM nondegradable sea urchin Δ 90-cyclin B for 60 min. The progress of cycling extracts was monitored by sperm morphology changes visualized by DAPI (4',6'-diamidino-2-phenylindole) staining and histone H1 kinase assays.

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Preparation of oocytes. *Xenopus* ovarian tissue was obtained surgically under tricaine anesthesia. Oocytes were treated with collagenase for 1 h and sorted manually. Stage VI oocytes were subjected to treatment with progesterone (160 ng/ml) or microinjection with Mos proteins (3.5 ng/oocyte) and were lysed in oocyte Ringer's solution (82.5 mM NaCl, 2.5 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 1 mM Na₂HPO₄, 5 mM HEPES, pH 7.8) supplemented with inhibitors (10 μg/ml leupeptin, 10 μg/ml chymostatin, 10 μg/ml pepstatin, 100 μg/ml cytochalasin B).

Mutagenesis and expression of recombinant Mos proteins. The coding region of *Xenopus* Mos was obtained by PCR from a clone provided by Monica Murakami and George Vande Woude (Van Andel Research Institute, Grand Rapids, MI) and subcloned into pGEX4T-1 (Amersham Biosciences, Piscataway, NJ), adding an N-terminal glutathione *S*-transferase (GST) tag and a Flag epitope tag. All conserved serine sites were individually or combinatorially mutated to alanine, glutamic acid, or aspartic acid using a QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). After mutagenesis, the Mos coding region was sequenced to ensure that no unintended mutations were introduced. GST-Flag-Mos wild-type and mutant proteins were then expressed in bacteria, lysed, and purified essentially as described in the Amersham GST purification manual.

Immunodepletions. Depletion of Mos or CK2β from extracts was accomplished by two sequential rounds of immunodepletion, carried out at 4°C with the relevant antibody (Mos, SC-86 [Santa Cruz Biotechnology] or CK2β [gift from Barbara Guerra, University of Southern Denmark, Odense, Denmark]) prebound to protein A-Sepharose beads (Sigma, St. Louis, MO). Mock depletions were carried out with rabbit immunoglobulin G.

Western blot analysis. Aliquots of egg extracts were diluted in sodium dodecyl sulfate (SDS) sample buffer and subjected to electrophoresis on 10% SDS polyacrylamide gels (usually low-bis gels with an acrylamide/bisacrylamide ratio of 100:1). Proteins were transferred to an Immobilon-P blotting membrane (Millipore, Billerica, MA), which was then blocked with 3% milk in Tris-buffered saline (20 mM Tris, 150 mM NaCl, pH 7.6) and incubated with primary antibody (Mos, SC-86 [Santa Cruz Biotechnology], 1:500 dilution; phospho-MAPK, no. 9106 [Cell Signaling Technology, Beverly, MA], 1:1,000 dilution; phospho-MEK, no. 9121 [Cell Signaling Technology, Beverly, MA], 1:1,000 dilution; CK2β, SC-12739 [Santa Cruz Biotechnology], 1:500 dilution; pS3-Mos, 1:1,000; pS105-Mos, 1:1,000; p42 MAPK DC3, 1:1,000) for 1 h. After the blots were washed, they were probed with a secondary antibody for detection by chemiluminescence. The ECL Advanced Detection System (Amersham) was used for detection of Mos. Immunoblotting was quantified by densitometry. Care was taken to ensure that the immunoblotting signal increased linearly with the amounts of Mos loaded (data not shown).

Phospho-specific antibodies. For the pS3-Mos-specific antibody, two sera were raised against the peptide MP-pS-PIPVERFL attached via a C-terminal cysteine residue to keyhole limpet hemocyanin. The sera were affinity purified on a phosphopeptide affinity matrix and then depleted of non-phospho-specific antibodies by adsorption on an unphosphorylated MPSPIPVERFL affinity matrix. For the pS105-Mos-specific antibody, sera were raised against the peptide SLASRQ-pS-FWAE-NH₂ conjugated to keyhole limpet hemocyanin through an N-terminal cysteine residue and purified as described above. Immunizations and affinity purifications were carried out by Zymed Laboratories (South San Francisco, CA).

Mos in vitro kinase assay. GST-Flag-Mos (final concentration, 30 nM) was added to 100 μl cycloheximide-treated interphase extracts with or without Δ90-cyclin B addition for 60 min. The extracts were then diluted with 100 μl extract buffer (EB; 80 mM β-glycerolphosphate, 20 mM EGTA, 15 mM MgCl₂, pH 7.4) and were incubated with anti-Flag beads (Sigma) with rocking for 1 h at 4°C. After three washes in EB containing 0.1% Nonidet P-40 and one wash in detergent-free EB, 25 μl of a reaction mixture containing 100 ng GST-MEK1-Flag, 10 μM cyclic AMP-dependent protein kinase inhibitor, 0.75 mM Na₃VO₄, 5 mM EGTA, 0.2 mM ATP, 13 mM HEPES-NaOH, pH 7.3, and 64 mM MgCl₂ was added, and the extracts were incubated at 30°C for 10 min. The reactions were stopped by the addition of SDS sample buffer. Proteins were subjected to electrophoresis through 10% SDS-polyacrylamide gels (acrylamide/bisacrylamide ratio, 29:1) and transferred to an Immobilon-P (Millipore) blotting membrane. Phosphorylated GST-MEK1-Flag was detected by Western blotting with anti-phospho-MEK antibody (9121; Cell Signaling Technology, Beverly, MA).

Histone H1 kinase assay. Assays were carried out as described by Dunphy and Newport (10), with minor modifications (41), and analyzed by autoradiography.

Identification of Mos phosphorylation sites by mass spectrometric analysis. GST-Flag-Mos was added to interphase egg extracts treated with or without 200 nM nondegradable sea urchin Δ90-cyclin B for 60 min. GST-Flag-Mos was then brought down by anti-Flag beads and resolved by 10% SDS-polyacrylamide gels

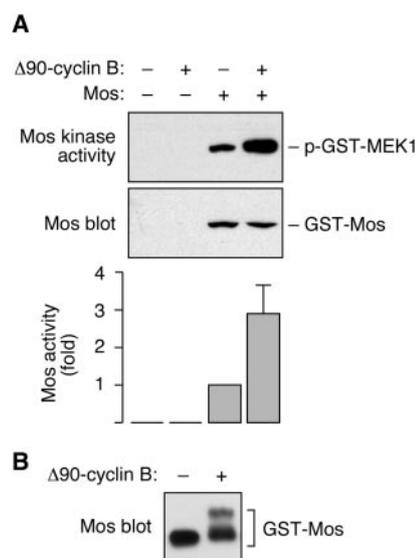


FIG. 1. Mitotic activation of Mos. (A) Activation of recombinant GST-Flag-Mos by Δ90-cyclin B-treated *Xenopus* egg extracts. The top blot shows Mos activity as indicated by an in vitro kinase assay using recombinant GST-MEK1 as a substrate and phospho-MEK1 immunoblotting to detect the amount of MEK1 phosphorylation. The bottom blot shows that equal levels of GST-Flag-Mos protein were present in the two samples. The bar graph shows pooled data from four independent experiments, with data expressed as means ± standard deviations. (B) Electrophoretic mobility shift of recombinant GST-Flag-Mos incubated with Δ90-cyclin B-treated *Xenopus* egg extracts. Samples were immunoprecipitated with Flag antibody and immunoblotted with Mos antibody.

(acrylamide/bisacrylamide ratio, 100:1). The gel was stained with Coomassie blue, and the gel bands were excised and sequenced at the Stanford mass spectrometric laboratory using microcapillary reverse-phase high-performance liquid chromatography nanoelectrospray tandem mass spectrometry (MS-MS) on a Micromass Q-ToF hybrid quadrupole time of flight liquid chromatographer-MS. Peptide sequence and phosphorylation analysis of Mos were determined by data searching against the Flag-*Xenopus*-Mos sequence using the Sequest algorithm in Bioworks.

Spindle checkpoint activation. Mock- or Mos-depleted CSF-arrested extracts were incubated for 30 min at 23°C with 15,000 sperm nuclei/μl and recombinant Mos wild-type or mutant protein as indicated in Fig. 6B, followed by another 30 min of incubation with 10 ng/μl nocodazole. Calcium chloride (1 mM) was then added to CSF extracts. Samples were taken immediately before the addition of calcium chloride and every 20 min thereafter for histone H1 kinase assays. Nuclear morphology was visualized by DAPI staining.

RESULTS

Activation of Mos in response to Δ90-cyclin B. Our previous work suggested that Mos activity could be regulated without changing the level of the Mos protein (44). To test this hypothesis further, we prepared a cycloheximide-treated interphase *Xenopus* egg extract, added 30 nM recombinant GST-Flag-Mos, and then incubated the extract for 60 min with either no added cyclin or sufficient Δ90-cyclin B to produce a sustained M-phase state. The Mos was pulled down with Flag antibody and subjected to a kinase assay using purified recombinant GST-MEK1 as a substrate and phospho-MEK1 immunoblotting to detect phosphorylation. As a control, we carried out Flag immunoprecipitations on extracts to which no GST-Flag-Mos had been added. As shown in Fig. 1A, in the absence of

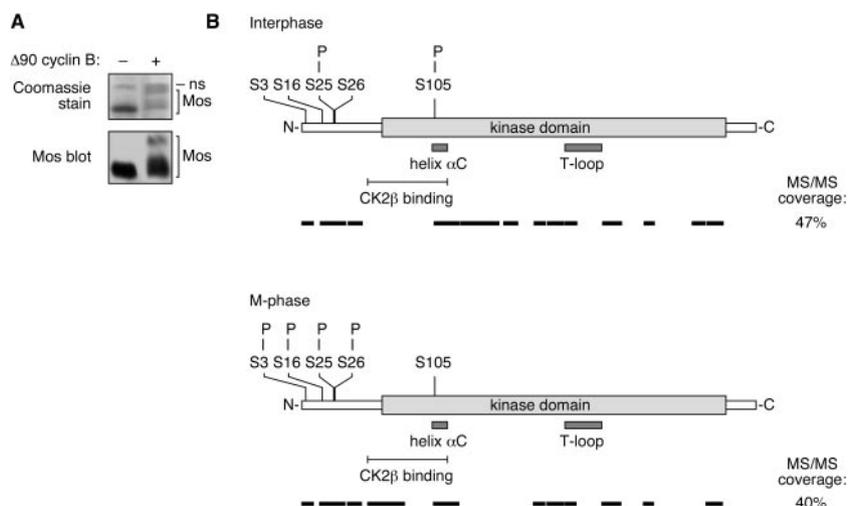


FIG. 2. Mos phosphorylation sites. (A) GST-Flag-Mos was treated with Δ90-cyclin B-treated *Xenopus* egg extracts or interphase extracts and subjected to immunoprecipitation with Flag antibody, followed by Coomassie blue staining (top) or immunoblotting with Mos antibody (bottom). “ns” designates a nonspecific band that ran just above Mos. (B) Schematic depiction of the Mos domain structure and the mapped phosphorylation sites. We have highlighted the kinase domain, helix αC, the region of Mos important for interaction with CK2β (21), and the T loop, or activation loop.

added GST-Flag-Mos, the Mos activities of the Flag immunoprecipitates were undetectable (lanes 1 and 2). However, in the presence of added GST-Flag-Mos, there was substantial Mos activity (lane 3), which increased approximately threefold after incubation with Δ90-cyclin B without any apparent change in Mos levels (lanes 3 and 4). The activation of Mos was accompanied by a modest increase in its apparent molecular mass that could be detected on low-bis polyacrylamide gels (Fig. 1B) but not on standard Laemmli gels (Fig. 1A).

MS-MS analysis of Mos phosphorylation. One straightforward mechanism for the cyclin-induced activation of Mos (Fig. 1A) and the shift in apparent molecular weight (Fig. 1B) would be a change in Mos phosphorylation. To test this idea and map the Mos phosphorylation sites, we treated GST-Flag-Mos with either interphase extract or M-phase extract and immunoprecipitated the GST-Flag-Mos with Flag antibody. The interphase-treated GST-Flag-Mos ran as a single band by SDS polyacrylamide gel electrophoresis, with a non-Mos band running just above it (Fig. 2A). The M-phase-treated GST-Flag-Mos ran as three bands, with the highest band running just below a non-Mos band (Fig. 2A). We excised the interphase GST-Flag-Mos band and the three M-phase Mos bands and subjected them to tryptic digestion and MS-MS analysis to look for tryptic phosphopeptides.

In total, five phosphorylation sites were identified (Fig. 2B). Two sites (Ser 25 and Ser 105) were phosphorylated in interphase Mos (Fig. 2B). Ser 25 remained phosphorylated in M-phase Mos (Fig. 2B) while Ser 105 became dephosphorylated, and three new phosphorylations (Ser 3, Ser 16, and Ser 26) appeared. Four sites (Ser 3, Ser 16, Ser 25, and Ser 26) were situated N terminal to the Mos kinase domain (Fig. 2B). One site (Ser 105) was situated at or near the N-terminal end of the αC helix, a secondary structure element in the N-terminal lobe of the kinase domain that plays a key role in the regulation of cyclin-dependent kinases and Src family tyrosine kinases (17, 19, 38).

Sites important for the electrophoretic mobility shift. To see which of the phosphorylations might be responsible for the electrophoretic mobility shift seen in M-phase wild-type Mos, we expressed and purified Mos mutants with each of the five phosphorylation sites individually mutated to alanine, incubated them with interphase or M-phase extracts, and then examined the mobilities of the Mos proteins by immunoblotting. We also examined the effects of mutation of three other conserved serine residues not found to be phosphorylation sites (Ser 18, Ser 57, and Ser 102). As shown in Fig. 3, the wild-type Mos protein and four of the mutants (S18A, S57A, S102A, and S105A) exhibited normal shifts. One protein (S25A) showed a slightly diminished shift (Fig. 3), two proteins (S16A and S26A) showed only a trace of a shift, and one protein (S3A) showed no shift. Thus, it appears that all four N-terminal phosphorylation sites (Ser 3, Ser 16, Ser 25, and Ser 26) contribute to the normal mitotic shift in Mos; mutation of any of the sites at least partially compromises the shift.

Sites important for Mos activation. We next set out to determine whether phosphorylation affected the kinase activity of Mos, using either interphase or M-phase extracts to activate wild-type or mutant Mos proteins and using recombinant MEK1 and phospho-MEK1 immunoblotting to determine the activities of the Mos proteins. First, we compared the activities of extract-treated Mos proteins to their activities after incuba-

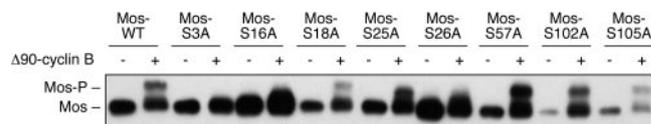


FIG. 3. Electrophoretic mobility shifts in Mos phosphorylation site mutants. Samples of wild-type GST-Flag-Mos (Mos-WT) and various Mos mutants were incubated with Δ90-cyclin B-treated *Xenopus* egg extracts or interphase extracts and were then immunoprecipitated with Flag antibody and immunoblotted with Mos antibody.

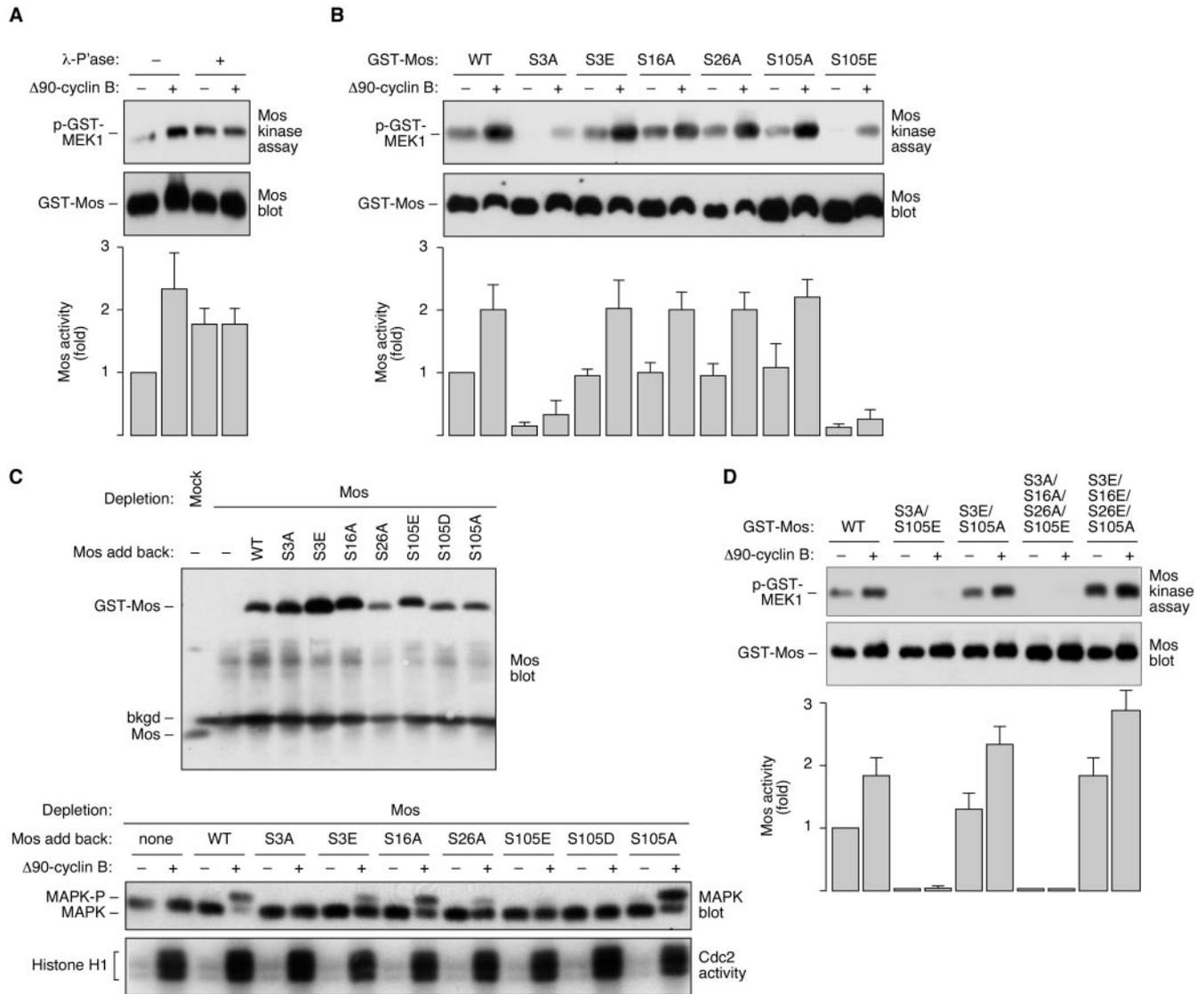


FIG. 4. Effects of phosphorylation on Mos activity. (A) Effect of λ phosphatase treatment on Mos activity. GST-Flag-Mos was treated with Δ 90-cyclin B-treated *Xenopus* egg extracts or interphase extracts and subjected to immunoprecipitation with Flag antibody. Samples were then treated with λ phosphatase (λ P'ase) or were mock phosphatase treated and were subjected to an in vitro kinase assay using recombinant GST-MEK1 as a substrate and phospho-MEK1 immunoblotting to detect the amount of MEK1 phosphorylation. The phospho-MEK1 and Mos blots are taken from one experiment. The bar graph shows data from three independent experiments expressed as means \pm standard deviations. (B) Activities of various phosphorylation site mutants. Kinase assays and blot analyses were carried out as described for panel A. The bar graph shows data from three independent experiments expressed as means \pm standard deviations. WT, wild type. (C) Reconstitution of the mitotic phosphorylation of p42 MAPK by adding Mos mutants back to Mos-depleted extracts. Extracts were depleted of Mos by two rounds of sequential immunodepletion. A nominal concentration of 30 nM wild-type GST-Flag-Mos and various Mos mutants was added back to the Mos-depleted extracts. The top panel shows a Mos blot of the extracts. The bottom panels show p42 MAPK blots and histone H1 kinase assay results for the Mos-depleted extracts and Mos-reconstituted extracts after incubation with or without Δ 90-cyclin B. Other studies showed that concentrations of Mos-S3A and Mos-S105E as high as 120 nM still failed to restore mitotic phosphorylation of p42 MAPK, as judged by the electrophoretic mobility shift (data not shown). bkgd, background band. (D) Activities of various multisite mutants. Kinase assays and blot analyses were carried out as described for panel A. The bar graph shows data from three independent experiments expressed as means \pm standard deviations.

tion with λ phosphatase. As shown in Fig. 4A, phosphatase treatment increased the activity of interphase Mos ($P = 0.02$ as calculated by a paired, one-tailed Student t test). Phosphatase treatment also possibly decreased the activity of M-phase Mos (Fig. 4A), although the statistical significance of the decrease was borderline ($P = 0.09$). This suggested that phosphorylation was capable of both positively and negatively regulating Mos activity.

Next, we examined the effects of alanine or glutamate substitutions on the activity of Mos. Substitution of alanine for either Ser 16 or Ser 26, two of the sites phosphorylated in mitotic Mos, had no effect on the basal activity or mitotic activation of Mos (Fig. 4B). Likewise, substitution of alanine for Ser 25 (a constitutive phosphorylation site) had no detectable effect on Mos activity (data not shown). However, Mos-S3A showed a markedly decreased activity, indicating that Ser

3 phosphorylation is required for Mos activity (Fig. 4B). Mos-S3E had a normal basal activity and normal activation in M phase. Taken together, these results suggest that the glutamate substitution successfully mimics the effects of phosphorylating Ser 3, but something in addition to the phosphorylation of Ser 3 contributes to the mitotic activation of Mos.

Mos-S105E exhibited a markedly decreased activity, consistent with the hypothesis that dephosphorylation of Ser 105 is necessary for mitotic activation of Mos (Fig. 4B). Mos-S105A had a normal basal activity and normal activation in M phase, suggesting that although Ser 105 dephosphorylation is required for the full activation of Mos, something other than the dephosphorylation of Ser 105, possibly the phosphorylation of Ser 3, is required as well.

We devised a second test of the kinase activities of the various Mos mutants. We depleted cycloheximide-treated, interphase *Xenopus* extracts of Mos, which abolished the extract's ability to activate p42 MAPK in response to treatment with $\Delta 90$ -cyclin B (Fig. 4C). We then supplemented the extract with sufficient wild-type Mos to largely restore the p42 MAPK response (30 nM) (Fig. 4C) and asked whether the mutant Mos proteins could also restore p42 MAPK response. The mutant proteins were all nominally 30 nM, based on Coomassie blue staining, although there was some variation in the apparent protein concentrations by immunoblotting (Fig. 4C, top). We found that the Mos-S16A and Mos-S26A proteins partially restored p42 MAPK responses (Fig. 4C), consistent with the data showing that these sites are not required for the mitotic activation of Mos (Fig. 4B). Mos-S3E also partially restored p42 MAPK activation, but Mos-S3A did not (Fig. 4C), even at concentrations of up to 120 nM (data not shown). This argues again that Ser 3 phosphorylation is required for the mitotic activation of Mos. Mos-S105A partially restored p42 MAPK activation, but Mos-S105E did not (Fig. 4C), even at concentrations of up to 120 nM (data not shown), again arguing that the dephosphorylation of Ser 105 is required for the mitotic activation of Mos.

We next asked whether a combination of two mutations—S3E, presumably mimicking the phosphorylation of Ser 3, and S105A, mimicking the dephosphorylation of Ser 105—would suffice to produce mitotic levels of Mos activity in interphase extracts. As shown in Fig. 4D, this was not the case; Mos-S3E/S105A had a normal basal activity and was activated normally in M-phase extracts. The same was true of the quadruple mutant Mos-S3E/S16E/S26E/S105A (Fig. 4D). In contrast, the double mutant Mos-S3A/S105E and the quadruple mutant Mos-S3A/S16A/S26A/S105E exhibited undetectably low activities in both interphase and M-phase extracts. This suggests that although Ser 3 phosphorylation and Ser 105 dephosphorylation are required for the mitotic activation of Mos, some third event is required as well.

Regulated binding of Mos to CK2 β . One possible mechanism to account for the activation of Mos-S3E/S105A in M-phase extracts (Fig. 4D) is provided by the work of Chen and coworkers (6, 7), who found that the regulatory (β) subunit of CK2 can bind to Mos and inhibit its activity. It seemed possible that the mitotic activation of Mos in $\Delta 90$ -cyclin B-treated extracts could be due to decreased Mos-CK2 β interaction, due either to changes in Mos, to changes in CK2 β , or to both. To test these possibilities, we immunodepleted interphase extracts

of CK2 β (Fig. 5A) and assessed the ability of the extracts to activate Mos in the absence and presence of $\Delta 90$ -cyclin B. As shown in Fig. 5A, the phosphorylation of p42 MAPK was accelerated by approximately 10 min in the CK2 β -depleted extracts, consistent with the hypothesis that CK2 β is a physiologically relevant Mos inhibitor (6, 7, 21). However, cyclin was still required for the phosphorylation of p42 MAPK in CK2 β -depleted extracts (Fig. 5A), arguing that cyclin does not act solely by releasing Mos from CK2 β .

Adding recombinant CK2 β back to CK2 β -depleted extracts restored the normal timing of p42 MAPK phosphorylation (Fig. 5A). In addition, we supplemented the extracts with additional CK2 β (Fig. 5B) and found that this increased the lag time between cyclin addition and p42 MAPK phosphorylation but did not render the extracts nonresponsive to cyclin (Fig. 5B).

Next, we examined whether the interaction between Mos and CK2 β was regulated or constitutive. GST-Flag-tagged CK2 β (20 nM) was incubated with GST-Flag-Mos (20 nM) in an interphase extract. Recombinant $\Delta 90$ -cyclin B was added to an aliquot to drive it into M phase. We then immunoprecipitated the GST-Flag-Mos with Mos antibodies. Control experiments showed that equal amounts of GST-Flag-Mos were pulled down from interphase and cyclin-treated extracts (Fig. 5C, lanes 7 versus 8, 9 versus 10, and 11 versus 12). The pulldowns were also probed for Mos-associated CK2 β . As shown in Fig. 5C, a small amount of CK2 β came down with Mos from interphase extracts (lane 7) but not from M-phase extracts (lane 8). To determine whether the dissociation was due to changes in Mos phosphorylation, we carried out the same pulldown experiment with recombinant GST-Flag-Mos-S3A/S16A/S26A/S105E to mimic the interphase state of Mos and with GST-Flag-Mos-S3E/S3E/S26E/S105A to mimic the M-phase state of Mos. Both of these mutants still associated with CK2 β during interphase and still dissociated from CK2 β during M phase (Fig. 5C, lanes 9 to 12). Thus, the dissociation of Mos from CK2 β appears not to be triggered by a change in Mos phosphorylation; a change in CK2 β (or possibly some other as-yet-unidentified factor) appears to be responsible for the dissociation.

Mos mutants as activators of oocyte maturation. To test the biological relevance of the M-phase changes in Mos phosphorylation, we injected immature oocytes with various Mos proteins and determined their relative abilities to induce maturation. We used a concentration of Mos (3.5 ng/oocyte) that produced near-maximal maturation for wild-type GST-Flag-Mos and no maturation for the least active Mos protein (Mos-S3A/S16A/S26A/S105E) and injected ~ 50 oocytes with each of seven purified Mos mutants. As shown in Fig. 6A, the biological potencies of the Mos proteins correlated well with their biochemical activities. Mos-S3E, Mos-S105A, and Mos-S3E/S16E/S26E/S105A were all similar to wild-type Mos in inducing maturation, whereas Mos-S3A, Mos-S105E, and Mos-S3A/S16A/S26A/S105E produced minimal levels of maturation. This is consistent with the hypothesis that the phosphorylation of Ser 3 and the dephosphorylation of Ser 105 are required for the activation of Mos as an inducer of meiotic maturation.

Mos function in the spindle assembly checkpoint. The demonstration that p42 MAPK function is important for the spindle assembly checkpoint in *Xenopus* egg extracts (24), together

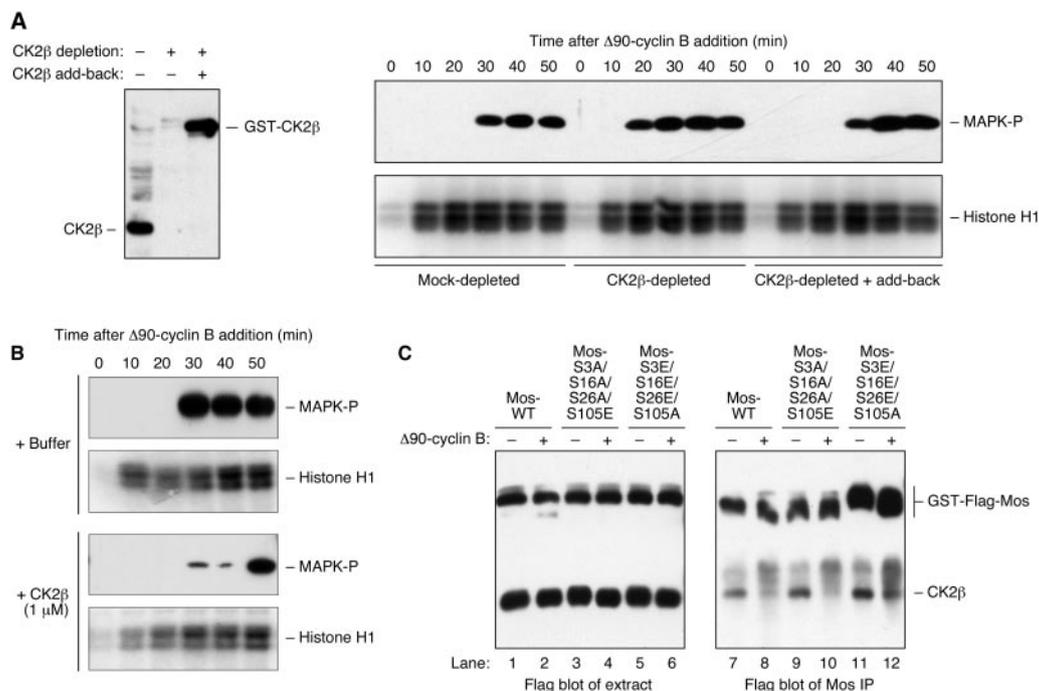


FIG. 5. Regulated dissociation of CK2 β from Mos. (A) Cyclin-dependent phosphorylation of p42 MAPK in CK2 β -depleted extracts. Extracts were subjected to two rounds of immunodepletion with nonspecific immunoglobulin G or anti-CK2 β . To one aliquot of the CK2 β -depleted extract, we added back recombinant CK2 β to restore normal levels of the protein. The resulting levels of CK2 β were determined by immunoblotting (left). The mock-depleted, CK2 β -depleted, and CK2 β -depleted/restored extracts were then treated with or without $\Delta 90$ -cyclin B and subjected to phospho-MAPK immunoblotting and histone H1 kinase assays (right). (B) Cyclin-dependent phosphorylation of p42 MAPK in CK2 β -supplemented extracts. Extracts were treated with buffer or CK2 β (1 μ M) for 30 min and then were treated with $\Delta 90$ -cyclin B. Samples were taken at various times and subjected to phospho-MAPK immunoblotting and histone H1 kinase assays. (C) Dissociation of CK2 β from Mos in M phase. Recombinant GST-Flag-CK2 β (20 nM) and either wild-type GST-Flag-Mos (Mos-WT) or one of two quadruple-phosphorylation-site Mos mutants (20 nM) were added to *Xenopus* egg extracts with or without $\Delta 90$ -cyclin B. Lysates were immunoblotted with Flag antibody (left) or were subjected to immunoprecipitation with Mos antibody, followed by immunoblotting with Flag antibody (right). Note that different Mos mutants were pulled down with differing efficiencies (e.g., compare lanes 9 and 10 with lanes 11 and 12), but cyclin treatment had no effect on the efficiency of the Mos pulldown (lane 7 versus lane 8; lane 9 versus lane 10; lane 11 versus lane 12).

with more-recent evidence implicating Mos in the M-phase activation of p42 MAPK in extracts (44), implied that Mos might be important for the spindle assembly checkpoint. To test this hypothesis, we incubated CSF-arrested *Xenopus* egg extracts with a high concentration of sperm chromatin (15,000 per μ l) in the absence or presence of nocodazole (10 ng/ μ l) and then added calcium. As shown in Fig. 6B, histone H1 kinase activity fell in the absence of nocodazole but was maintained at high levels in the presence of nocodazole, corroborating previous work (24). Moreover, depleting Mos abrogated the extract's ability to maintain high levels of H1 kinase activity in the presence of nocodazole, and adding back recombinant wild-type Mos restored the H1 kinase activity to M-phase levels (Fig. 6B). This demonstrates that Mos is required for the spindle assembly checkpoint in extracts.

These findings also provide us with a second context in which to assess the biological activities of Mos mutants. As shown in Fig. 6B, neither Mos-S3A nor Mos-S105E was able to maintain high levels of H1 kinase activity in Mos-depleted extracts treated with sperm chromatin, nocodazole, and calcium. Thus, both oocyte maturation (Fig. 6A) and the spindle assembly checkpoint (Fig. 6B) require the presence of an activable form of Mos.

Phosphorylation of Ser 3 and Ser 105 in oocytes and extracts. We next set out to determine whether the phosphorylation of Ser 3 and Ser 105 was regulated in vivo. We obtained phospho-specific antibodies against phosphoserine 3 (pS3-Mos) and phosphoserine 105 (pS105-Mos). As shown in Fig. 7A, the pS3-Mos antibody recognized M-phase wild-type Mos but not interphase wild-type Mos. The pS3-Mos antibody did not recognize Mos-S3A but did recognize M-phase Mos-S105A (Fig. 7A). Conversely, the pS105-Mos antibody recognized interphase wild-type Mos but not M-phase wild-type Mos and recognized interphase Mos-S3A but not interphase Mos-S105A (Fig. 7A). This establishes the specificities of the two antibodies.

We then looked for changes in Mos levels and Mos phosphorylation during *Xenopus* oocyte maturation. In the experiment whose results are shown in Fig. 7B, H1 kinase activity first appeared about 2 h after progesterone treatment. Mos was first detectable 1 h after progesterone treatment and reached maximal levels by 4 to 5 h (Fig. 7B), consistent with previous reports (2, 11, 32, 34). The time course of Ser 3 phosphorylation was similar, reaching maximal levels by 4 to 5 h, but there was no detectable pS105-Mos signal (Fig. 7B). These findings indicate that fairly early on in maturation, the balance of ki-

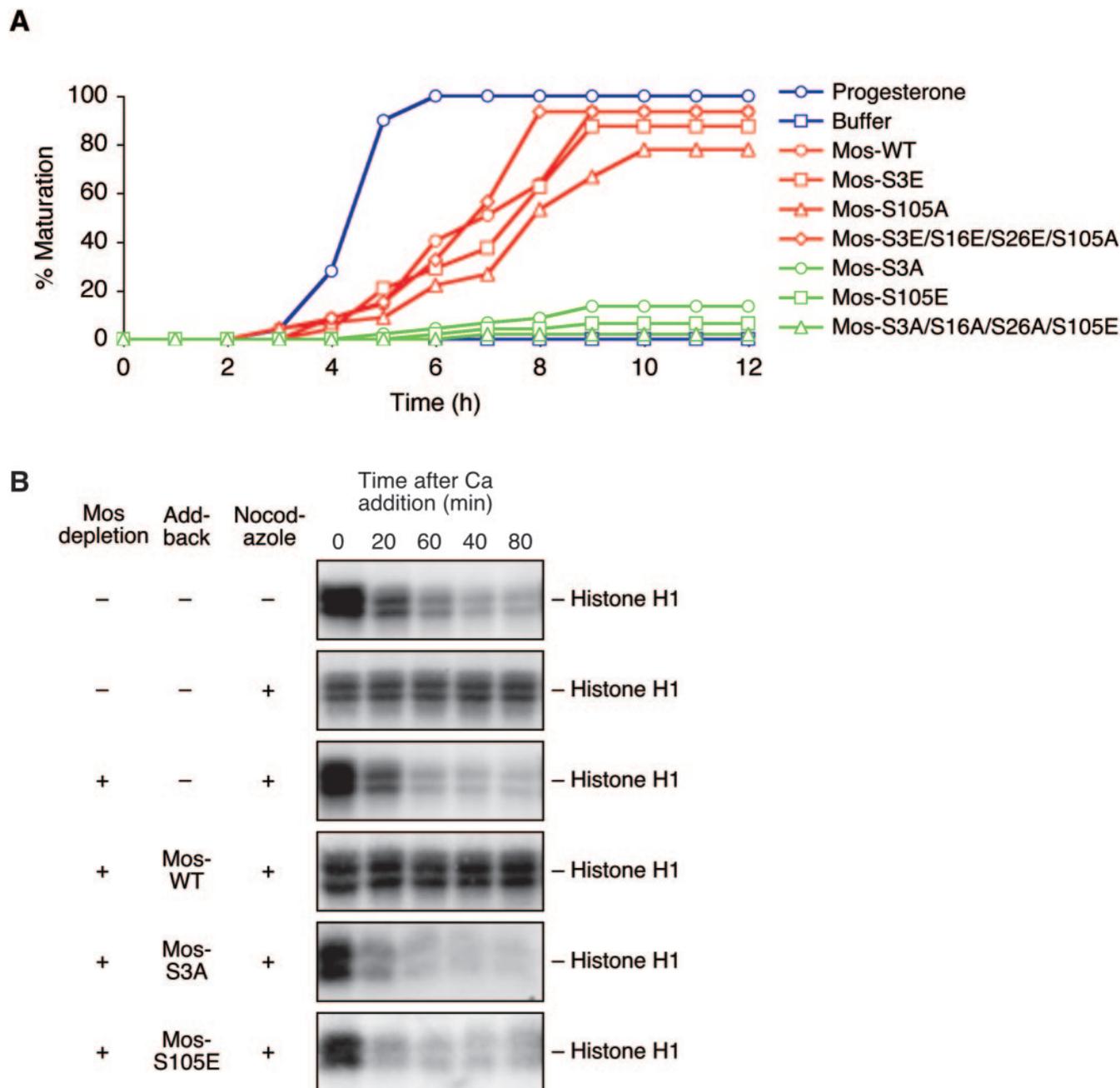


FIG. 6. Biological function of Mos phosphorylation site mutants. (A) Oocyte maturation. Oocytes were incubated with progesterone or buffer or were microinjected with one of the seven GST-Flag-Mos proteins as indicated. Maturation was scored as a function of time, using the appearance of a white dot at the animal pole of the oocyte as an indicator of maturation. (B) Spindle assembly checkpoint. CSF-arrested *Xenopus* egg extracts were subjected to two rounds of Mos immunodepletion or mock depletion. Sperm chromatin was added at a concentration of 15,000 per μl , and calcium (1 mM) was added in the absence and presence of nocodazole (10 ng/ μl). Histone H1 kinase assays were monitored as functions of time. WT, wild type.

nase and phosphatase activities favors the phosphorylation of Ser 3 and the dephosphorylation of Ser 105 (Fig. 2B and 7B).

We also followed Mos levels and phosphorylation after treating dejellied eggs with calcium ionophore to parthenogenetically activate them. As shown in Fig. 7C, Mos levels began to fall within 20 min of ionophore treatment and continued to fall through 60 min. This gradual decrease in Mos levels resulted in a more temporally abrupt decrease in p42 MAPK

phosphorylation, which appeared to be nearly complete within 30 min of ionophore treatment. This suggested that something in addition to the gradual decrease in Mos levels contributed to the inactivation of the p42 MAPK cascade, and indeed the inactivation of p42 MAPK corresponded temporally to the dephosphorylation of Mos at Ser 3 and the phosphorylation of Mos at Ser 105 (Fig. 7C). Thus, it appears that the inactivation of the p42 MAPK cascade after egg activation results not only

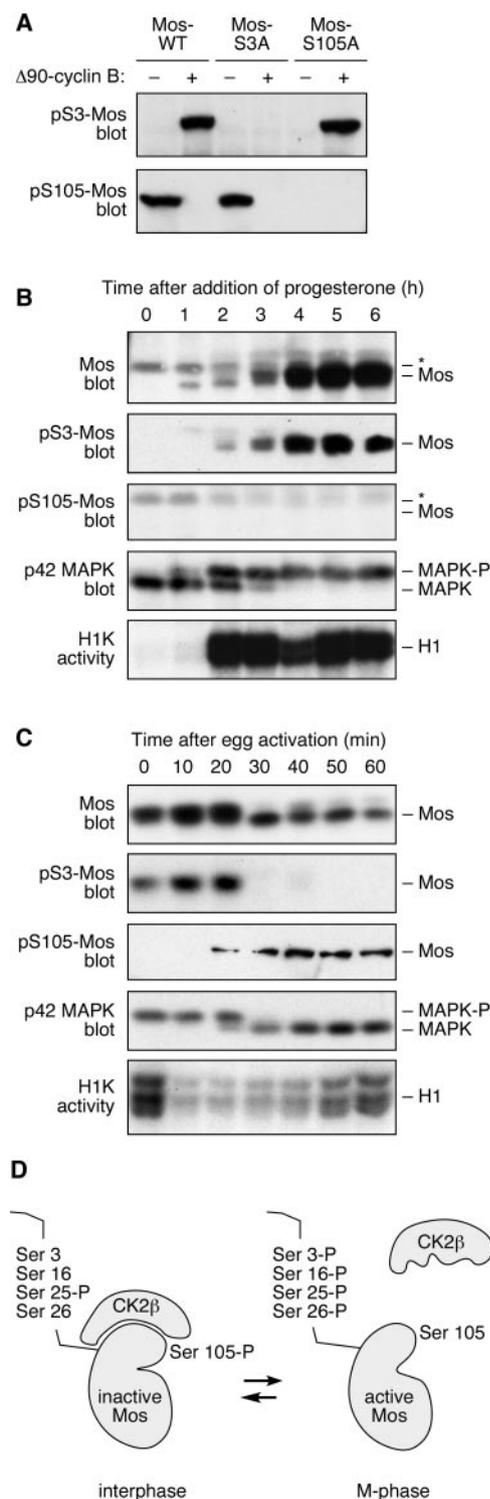


FIG. 7. Phosphorylation of Mos at Ser 3 and Ser 105 during oocyte maturation and after egg activation. (A) Specificities of the pS3-Mos and pS105-Mos antibodies. Wild-type (WT) or mutant GST-Flag-Mos was treated with $\Delta 90$ -cyclin B-treated *Xenopus* egg extracts or interphase extracts and subjected to immunoprecipitation with Flag antibody, followed by immunoblotting with pS3-Mos and pS105-Mos antibodies. (B) Mos accumulation and phosphorylation during oocyte maturation. Oocytes were treated with progesterone for various lengths of time. Lysates were subjected to immunoblotting with Mos, pS3-Mos, and pS105-Mos antibodies. The asterisks denote nonspecific protein bands in

from a decrease in Mos levels but also from a decrease in Ser 3 phosphorylation and an increase in Ser 105 phosphorylation.

DISCUSSION

Mos phosphorylation sites. Here we have identified five phosphorylation sites in *Xenopus* Mos: Ser 3, Ser 16, Ser 25, Ser 26, and Ser 105. Each of the five sites is relatively well conserved among vertebrate Mos proteins, although Ser 26 is mutated to a nonphosphorylatable residue (Ile) in mouse Mos and viral Mos, and none of the N-terminal phosphorylation sites (Ser 3 through Ser 26) is present in the starfish (*Asterina*) and *Drosophila* Mos orthologs. Three of the phosphorylation sites are candidates for Cdk phosphorylation (Ser 3, MPS³PIP; Ser 16, DLS¹⁶PSI; Ser 26, CSS²⁶PLE). One site is a candidate Plk1 site (Ser 25, CS²⁵SPLE). The last site (Ser 105, RRS¹⁰⁵FW) is weakly predicted to be a potential basicophilic kinase site by the Scansite algorithm (29). No phosphorylation sites were found in the Mos activation loop, a region whose phosphorylation is often important for protein kinase activation.

The phosphorylation of Ser 3 has been previously implicated in Mos stability (28, 37). Here we show the importance of Ser 3 for Mos activity as well. Replacing Ser 3 with a nonphosphorylatable alanine residue decreased the activity of extract-treated GST-Flag-Mos (Fig. 4) and markedly decreased the ability of recombinant Mos to stimulate oocyte maturation (Fig. 6A) and to support the spindle assembly checkpoint (Fig. 6B). The decreased biological activity of Mos-S3A was not apparent in early studies of Mos phosphorylation (13, 28); however, two subsequent studies are more consistent with the present findings (5, 22).

In addition, we have demonstrated that the dephosphorylation of Ser 105 is important for the activation of Mos. We found that Mos-S105A was normal in terms of its biochemical and biological activities (Fig. 4 and 6), in contrast to the low biological activity of Mos-S105A reported by Freeman et al. (13). The source of this discrepancy is uncertain, but the normal activity we found for Mos-S105A was consistent between the biochemical measures of Mos activity (Fig. 4B) and two biological measures of Mos activity (Fig. 6A and B). In contrast to the normal activity of Mos-S105A, we found that Mos-S105E was defective for activation (Fig. 4 and 6). Together, these studies argue that the dephosphorylation of Ser 105 is required for the activation of Mos.

Finally, we confirm previous studies implicating CK2 β as a physiologically important inhibitor of Mos and furthermore establish that CK2 β is induced to dissociate from Mos during M phase (Fig. 5). The dissociation appears to be due to some M-phase-induced change in CK2 β rather than a change in Mos phosphorylation, since two Mos quadruple

the Mos blots, p42 MAPK blots and H1 kinase (H1K) assay results are shown for comparison. (C) Mos degradation and phosphorylation after egg activation. Dejellied eggs were treated with calcium ionophore and blotted for Mos, pS3-Mos, and pS105-Mos. p42 MAPK blots and H1 kinase assay results are shown for comparison. (D) Schematic depiction of the changes in Mos phosphorylation and CK2 β association between interphase and M phase.

Ala/Glu mutants still dissociated normally from CK2 β during M phase (Fig. 5C).

Thus, the activation of Mos during M phase depends upon multiple regulatory events: the phosphorylation of Ser 3, the dephosphorylation of Ser 105, and the dissociation of CK2 β . These findings are summarized in Fig. 7D.

Phosphorylation of the N-terminal regulatory domain of Mos. Ser 3 is part of a cluster of N-terminal phosphorylation sites that regulate the electrophoretic mobility of Mos (Fig. 3). This suggests that the phosphorylation of this region exerts some general electrostatic effect on the conformation of the N terminus of Mos or the interaction of the N terminus with proteins, lipids, and/or detergents (SDS). Ser 3 was the only one of this cluster of residues whose mutation affected Mos activity, and the S3A mutation also produced the most pronounced effect on the electrophoretic mobility of Mos (Fig. 3 and 4B).

Dephosphorylation of helix α C. Ser 105 is situated within the kinase domain of Mos. By sequence alignment, Ser 105 is a part of kinase subdomain III (16) and it is predicted to sit at or near the N terminus of helix α C. This helix is known to play a critical role in the activation of Cdk2 and Src family tyrosine kinases (19, 38, 43). In the case of Cdk2, cyclin binding causes a rotation of α C (which in the case of Cdks is the PSTAIRE helix), which allows Glu 51 to pull Lys 33 away from the catalytic Asp 145, freeing up this last residue for catalysis. Furthermore, cyclin binding causes α C to bump into the activation loop (also called the A loop or T loop), pushing it out of the way of the catalytic cleft and allowing protein substrates to have access to the cleft (19). These rearrangements are still not sufficient for full activation of Cdk2—that requires the additional phosphorylation of Thr 160—but they contribute in an important way to the overall activation (9, 15, 33). In the case of Src family kinases, activation is brought about by a similar reorientation of α C, with similar consequences—the binding of the α C Glu 310 to Lys 295 and a displacement of the activation loop—though the reorientation of α C is brought about by a completely different mechanism, the dissociation of the Src SH2 domain from a C-terminal phosphotyrosine residue and the dissociation of the Src SH3 domain from a polyproline linker sequence (38, 43).

The current findings suggest that the activation of Mos depends upon similar reorientations of α C, in this case triggered by a third distinct mechanism, the dephosphorylation of Ser 105. We conjecture that this dephosphorylation results in the binding of Glu 109 to Lys 90 and/or the reorientation of the Mos activation loop. It is interesting to note that an activating mutation in the v-Mos protein has been mapped to a nearby residue, Arg 114, which is predicted to sit at the other (C-terminal) end of helix α C (31). Replacement of this residue with glycine increases the biochemical and biological activities of Mos (31). It is easy to imagine that the arginine-to-glycine substitution at the C-terminal end of helix α C and the dephosphorylation of Ser 105 at the N-terminal end of helix α C might have similar structural consequences.

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REFERENCES

1. Abrieu, A., D. Fisher, M. N. Simon, M. Doree, and A. Picard. 1997. MAPK inactivation is required for the G2 to M-phase transition of the first mitotic cell cycle. *EMBO J.* **16**:6407–6413.
2. Andrésson, T., and J. V. Ruderman. 1998. The kinase Eg2 is a component of the *Xenopus* oocyte progesterone-activated signaling pathway. *EMBO J.* **17**:5627–5637.
3. Bitangcol, J. C., A. S. Chau, E. Stadnick, M. J. Lohka, B. Dicken, and E. K. Shibuya. 1998. Activation of the p42 mitogen-activated protein kinase pathway inhibits Cdc2 activation and entry into M-phase in cycling *Xenopus* egg extracts. *Mol. Biol. Cell* **9**:451–467.
4. Castro, A., M. Peter, L. Magnaghi-Jaulin, S. Vigneron, S. Galas, T. Lorca, and J. C. Labbe. 2001. Cyclin B/cdc2 induces c-Mos stability by direct phosphorylation in *Xenopus* oocytes. *Mol. Biol. Cell* **12**:2660–2671.
5. Chen, M., and J. A. Cooper. 1995. Ser-3 is important for regulating Mos interaction with and stimulation of mitogen-activated protein kinase. *Mol. Cell. Biol.* **15**:4727–4734.
6. Chen, M., and J. A. Cooper. 1997. The beta subunit of CKII negatively regulates *Xenopus* oocyte maturation. *Proc. Natl. Acad. Sci. USA* **94**:9136–9140.
7. Chen, M., D. Li, E. G. Krebs, and J. A. Cooper. 1997. The casein kinase II beta subunit binds to Mos and inhibits Mos activity. *Mol. Cell. Biol.* **17**:1904–1912.
8. Colledge, W. H., M. B. Carlton, G. B. Udy, and M. J. Evans. 1994. Disruption of c-mos causes parthenogenetic development of unfertilized mouse eggs. *Nature* **370**:65–68.
9. Connell-Crowley, L., M. J. Solomon, N. Wei, and J. W. Harper. 1993. Phosphorylation independent activation of human cyclin-dependent kinase 2 by cyclin A in vitro. *Mol. Biol. Cell* **4**:79–92.
10. Dunphy, W. G., and J. W. Newport. 1989. Fission yeast p13 blocks mitotic activation and tyrosine dephosphorylation of the *Xenopus* cdc2 protein kinase. *Cell* **58**:181–191.
11. Dupre, A., C. Jessus, R. Ozon, and O. Haccard. 2002. Mos is not required for the initiation of meiotic maturation in *Xenopus* oocytes. *EMBO J.* **21**:4026–4036.
12. Ferrell, J. E., Jr. 1999. *Xenopus* oocyte maturation: new lessons from a good egg. *Bioessays* **21**:833–842.
13. Freeman, R. S., A. N. Meyer, J. Li, and D. J. Donoghue. 1992. Phosphorylation of conserved serine residues does not regulate the ability of mos protein kinase to induce oocyte maturation or function as cytostatic factor. *J. Cell Biol.* **116**:725–735.
14. Guadagno, T. M., and J. E. Ferrell, Jr. 1998. Requirement for MAPK activation for normal mitotic progression in *Xenopus* egg extracts. *Science* **282**:1312–1315.
15. Hagopian, J. C., M. P. Kirtley, L. M. Stevenson, R. M. Gergis, A. A. Russo, N. P. Pavletich, S. M. Parsons, and J. Lew. 2001. Kinetic basis for activation of Cdk2/CyclinA by phosphorylation. *J. Biol. Chem.* **276**:275–280.
16. Hanks, S. K., and A. M. Quinn. 1991. Protein kinase catalytic domain sequence database: identification of conserved features of primary structure and classification of family members. *Methods Enzymol.* **200**:38–62.
17. Harrison, S. C. 2003. Variation on an Src-like theme. *Cell* **112**:737–740.
18. Hashimoto, N., N. Watanabe, Y. Furuta, H. Tamemoto, N. Sagata, M. Yokoyama, K. Okazaki, M. Nagayoshi, N. Takeda, Y. Ikawa, et al. 1994. Parthenogenetic activation of oocytes in c-mos-deficient mice. *Nature* **370**:68–71.
19. Jeffrey, P. D., A. A. Russo, K. Polyak, E. Gibbs, J. Hurwitz, J. Massague, and N. P. Pavletich. 1995. Mechanism of CDK activation revealed by the structure of a cyclinA-CDK2 complex. *Nature* **376**:313–320.
20. Kajjura-Kobayashi, H., N. Yoshida, N. Sagata, M. Yamashita, and Y. Nagahama. 2000. The Mos/MAPK pathway is involved in metaphase II arrest as a cytostatic factor but is neither necessary nor sufficient for initiating oocyte maturation in goldfish. *Dev. Genes Evol.* **210**:416–425.
21. Lieberman, S. L., and J. V. Ruderman. 2004. CK2 beta, which inhibits Mos function, binds to a discrete domain in the N-terminus of Mos. *Dev. Biol.* **268**:271–279.
22. Matten, W. T., T. D. Copeland, N. G. Ahn, and G. F. Vande Woude. 1996. Positive feedback between MAP kinase and Mos during *Xenopus* oocyte maturation. *Dev. Biol.* **179**:485–492.
23. Mendez, R., L. E. Hake, T. Andresson, L. E. Littlepage, J. V. Ruderman, and J. D. Richter. 2000. Phosphorylation of CPE binding factor by Eg2 regulates translation of c-mos mRNA. *Nature* **404**:302–307.
24. Minshull, J., H. Sun, N. K. Tonks, and A. W. Murray. 1994. A MAP kinase-dependent spindle assembly checkpoint in *Xenopus* egg extracts. *Cell* **79**:475–486.
25. Murray, A. W. 1991. Cell cycle extracts. *Methods Cell Biol.* **36**:581–605.

26. Nebreda, A. R., and I. Ferby. 2000. Regulation of the meiotic cell cycle in oocytes. *Curr. Opin. Cell Biol.* **12**:666–675.
27. Nishizawa, M., N. Furuno, K. Okazaki, H. Tanaka, Y. Ogawa, and N. Sagata. 1993. Degradation of Mos by the N-terminal proline (Pro2)-dependent ubiquitin pathway on fertilization of *Xenopus* eggs: possible significance of natural selection for Pro2 in Mos. *EMBO J.* **12**:4021–4027.
28. Nishizawa, M., K. Okazaki, N. Furuno, N. Watanabe, and N. Sagata. 1992. The 'second-codon rule' and autophosphorylation govern the stability and activity of Mos during the meiotic cell cycle in *Xenopus* oocytes. *EMBO J.* **11**:2433–2446.
29. Obenauer, J. C., L. C. Cantley, and M. B. Yaffe. 2003. Scansite 2.0: proteome-wide prediction of cell signaling interactions using short sequence motifs. *Nucleic Acids Res.* **31**:3635–3641.
30. Pascreau, G., J. G. Delcros, J. Y. Cremet, C. Prigent, and Y. Arlot-Bonnemains. 2005. Phosphorylation of maskin by Aurora-A participates in the control of sequential protein synthesis during *Xenopus laevis* oocyte maturation. *J. Biol. Chem.* **280**:13415–13423.
31. Puls, A., T. Proikas-Cezanne, B. Marquardt, F. Propst, and S. Stabel. 1995. Kinase activities of c-Mos and v-Mos proteins: a single amino acid exchange is responsible for constitutive activation of the 124 v-Mos kinase. *Oncogene* **10**:623–630.
32. Roy, L. M., O. Haccard, T. Izumi, B. G. Lattes, A. L. Lewellyn, and J. L. Maller, J. L. 1996. Mos proto-oncogene function during oocyte maturation in *Xenopus*. *Oncogene* **12**:2203–2211.
33. Russo, A. A., P. D. Jeffrey, and N. P. Pavletich. 1996. Structural basis of cyclin-dependent kinase activation by phosphorylation. *Nat. Struct. Biol.* **3**:696–700.
34. Sagata, N., I. Daar, M. Oskarsson, S. D. Showalter, and G. F. Vande Woude. 1989. The product of the *mos* proto-oncogene as a candidate "initiator" for oocyte maturation. *Science* **245**:643–646.
35. Sagata, N., M. Oskarsson, T. Copeland, J. Brumbaugh, and G. F. Vande Woude. 1988. Function of c-mos proto-oncogene product in meiotic maturation in *Xenopus* oocytes. *Nature* **335**:519–525.
36. Sagata, N., N. Watanabe, G. F. Vande Woude, and Y. Ikawa. 1989. The *c-mos* proto-oncogene product is a cytostatic factor responsible for meiotic arrest in vertebrate eggs. *Nature* **342**:512–518.
37. Sheng, J., A. Kumagai, W. G. Dunphy, and A. Varshavsky. 2002. Dissection of c-MOS degron. *EMBO J.* **21**:6061–6071.
38. Sicheri, F., and J. Kuriyan. 1997. Structures of Src-family tyrosine kinases. *Curr. Opin. Struct. Biol.* **7**:777–785.
39. Smythe, C., and J. W. Newport. 1991. Systems for the study of nuclear assembly, DNA replication, and nuclear breakdown in *Xenopus laevis* egg extracts. *Methods Cell Biol.* **35**:449–468.
40. Verlhac, M. H., J. Z. Kubiak, M. Weber, G. Geraud, W. H. Colledge, M. J. Evans, and B. Maro. 1996. Mos is required for MAP kinase activation and is involved in microtubule organization during meiotic maturation in the mouse. *Development* **122**:815–822.
41. Walter, S. A., T. M. Guadagno, and J. E. Ferrell, Jr. 1997. Induction of a G2-phase arrest in *Xenopus* egg extracts by activation of p42 MAP kinase. *Mol. Biol. Cell* **8**:2157–2169.
42. Watanabe, N., T. Hunt, Y. Ikawa, and N. Sagata. 1991. Independent inactivation of MPF and cytostatic factor (Mos) upon fertilization of *Xenopus* eggs. *Nature* **352**:247–248.
43. Xu, W., A. Doshi, M. Lei, M. J. Eck, and S. C. Harrison. 1999. Crystal structures of c-Src reveal features of its autoinhibitory mechanism. *Mol. Cell* **3**:629–638.
44. Yue, J., and J. E. Ferrell, Jr. 2004. Mos mediates the mitotic activation of p42 MAPK in *Xenopus* egg extracts. *Curr. Biol.* **14**:1581–1586.