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Raf-1 Protein Kinase Is Important for Progesterone-Induced *Xenopus* Oocyte Maturation and Acts Downstream of mos

ANTHONY J. MUSLIN, ANGUS M. MACNICOL, AND LEWIS T. WILLIAMS*

Program of Excellence in Molecular Biology, Howard Hughes Medical Institute, Cardiovascular Research Institute, Department of Medicine, University of California, San Francisco, California 94143-0130

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In somatic cells, the Raf-1 serine/threonine protein kinase is activated by several polypeptide growth factors. We investigated the role of Raf-1 in progesterone-induced meiotic maturation of *Xenopus laevis* oocytes. Raf-1 enzymatic activity and phosphorylation (reflected by a mobility shift on sodium dodecyl sulfate gels) were increased in oocytes following progesterone stimulation. The increase in Raf-1 activity was concurrent with an elevation in the activity of mitogen-activated protein (MAP) kinase. When RNA encoding an oncogenic form of Raf-1 (v-Raf) was injected into immature oocytes, MAP kinase mobility shift, germinal vesicle breakdown, and histone H1 phosphorylation increased markedly. When RNA encoding a dominant-negative version of Raf-1 was injected, progesterone-induced oocyte maturation was blocked. When RNA encoding *Xenopus* mos (mos^{xe}) was injected into oocytes, Raf-1 and MAP kinase mobility shifts were observed after several hours. Also, when antisense mos^{xe} oligonucleotides were injected into oocytes, progesterone-induced Raf-1 and MAP kinase mobility shifts were blocked. Finally, when antisense mos^{xe} oligonucleotides were coinjected with v-Raf RNA into oocytes, histone H1 kinase activation, germinal vesicle breakdown, and MAP kinase mobility shift occurred. These findings suggest that Raf-1 activity is required for progesterone-induced oocyte maturation and that Raf-1 is downstream of mos^{xe} activity.

The Raf-1 serine/threonine protein kinase is activated in cultured cells by a variety of growth factors (18, 19, 21). Raf-1 activity is necessary for somatic cell transformation by a variety of oncogenes and is also necessary for growth factor-induced proliferation of certain cultured cells (9). Raf-1 consists of an amino-terminal regulatory domain and a carboxyl-terminal kinase domain. An oncogenic form of Raf-1, v-Raf, has an amino-terminal deletion of much of the putative regulatory domain (22, 26).

When wild-type Raf-1 is activated, it has the ability to phosphorylate and activate mitogen-activated protein (MAP) kinase kinase in vitro and in cultured cells (2, 6, 12). This activity of Raf-1 on MAP kinase kinase is thought to be an important step in mitogenic signal transduction. The Raf-1 amino acid sequence is highly conserved among vertebrate species and is closely related to the *Drosophila* Draf sequence (16). Recently, it was demonstrated that Raf-1 mRNA is present in *Xenopus laevis* oocytes and embryos (13). The importance of Raf-1 in somatic cell signal transduction led us to hypothesize that Raf-1 also plays an important role in the regulation of meiosis in oocytes.

The maturation of *Xenopus* oocytes has been used previously as a model system for the study of cell cycle regulation. The process of maturation is triggered by exposure to progesterone, which causes the oocytes to undergo a series of biochemical alterations, including the synthesis and activation of *Xenopus* mos (mos^{xe}) protein kinase (23, 24). The progesterone-induced signal cascade culminates in the completion of meiosis I and the progression to metaphase of meiosis II (25). Entry into meiosis I is associated with germinal vesicle breakdown (GVBD) and correlates with histone H1 kinase activation and MAP kinase phosphorylation (reflected by a mobility shift on sodium dodecyl sulfate [SDS] gels) (3, 20).

Given the importance of Raf-1 in growth factor-stimulated mitogenesis of somatic cells, we speculated that Raf-1 may play an important role in progesterone-induced oocyte meiotic maturation. To test this hypothesis, we have used an oncogenic Raf-1 cDNA (v-Raf) and a dominant-negative Raf-1 cDNA (9, 15). Employing such constructs, we have determined that Raf-1 activity is required for progesterone-induced *Xenopus* oocyte maturation and that Raf-1 activity is downstream of mos^{xe}.

MATERIALS AND METHODS

Antibodies. We used a previously described (15) antipeptide mouse monoclonal antibody against the last 12 amino acids of human Raf-1. A commercial (Zymed) anti-MAP kinase mouse monoclonal antibody was used for Western immunoblotting. The mouse monoclonal antibody used for MAP kinase immunoprecipitation was kindly obtained from Jonathan A. Cooper (Fred Hutchinson Cancer Research Center). The Raf-1 and MAP kinase antibodies used for immunoprecipitations recognized the phosphorylated and unphosphorylated forms of the proteins with equal affinity.

Plasmid constructs and RNA transcription. The v-Raf construct was made by site-directed mutagenesis (Amersham), using a human full-length Raf-1 cDNA as a template. An amino-terminal deletion of the protein was made by introducing an *Nco*I site into the coding sequence at amino acid 320. This converted the proline to methionine (P320M). The v-Raf construct was excised by digestion with *Nco*I and *Xba*I (from the 3′ polylinker of the vector) and subcloned into pSP64T, a vector which includes 5′ and 3′ *Xenopus* β-globin untranslated sequence (11). Additionally, a double-stranded oligonucleotide encoding the KT3 peptide TPPP-EPET (14) and an in-frame termination codon was inserted into the construct at the 3′ end of the v-Raf coding sequence.

The NAF construct consists of an ATP binding site mutation in the human c-Raf-1 catalytic domain and was

* Corresponding author.

made by performing M13 site-directed mutagenesis, converting lysine 375 to methionine (K375M) as described previously (15). The NAF and wild-type Raf-1 cDNAs were inserted into the pSP64T vector, and the constructs were modified by insertion of an oligonucleotide encoding the KT3 peptide at the 3′ ends of the coding regions.

The full-length mos^{xe} cDNA construct and the mos^{xe} antisense oligonucleotides were generous gifts of Daniel J. Donoghue (University of California, San Diego) (4, 5, 7).

RNA containing a 5′-GpppG cap (Pharmacia LKB Biotechnology Inc.) was made by using linearized plasmid with the SP6 RNA polymerase (Boehringer Mannheim) as described previously (17) and resuspended in water.

Oocyte injections. Large oocytes (Dumont stage VI) were removed from adult female frogs by using established techniques. Oocytes were manually dissected and collagenase treated (Sigma type II, 1 mg/ml). Oocytes were maintained in 1× modified Barth's saline with *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) [MBSH; 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 10 mM HEPES (pH 7.4)], bovine serum albumin (BSA; 1 mg/ml), Ficoll 400 (1 mg/ml), and antibiotics (27). Each oocyte was injected with 10 ng of RNA (except for the NAF/wild-type-injected oocytes, which were injected with 10 ng of each type of RNA for a total of 20 ng) in 1× MBSH with added Ficoll, BSA, and antibiotics as described previously (1). Oocytes were incubated for 24 h at 18°C, and then some were stimulated with progesterone (2 μg/ml).

Isolation and analysis of protein. Clarified oocyte lysates were made by using 10 μl of ice-cold Nonidet P-40 lysis buffer (137 mM NaCl, 50 mM NaF, 0.5% Nonidet P-40, 10 mM Tris-HCl [pH 7.5], 2 mM phenylmethylsulfonyl fluoride, 0.2 U of aprotinin per ml, 25 mM leupeptin) per oocyte. Insoluble material and lipid were separated by centrifugation at 13,000 × *g* for 10 min at 4°C. Lysates were loaded onto SDS-polyacrylamide gels. Equal amounts of total protein were loaded into all lanes. Proteins were transferred to 0.2 mM nitrocellulose paper (Schleicher & Schuell). The blots were blocked with 2% nonfat dry milk and were treated overnight in primary antibody at 4°C. Bands were visualized by using an alkaline phosphatase-conjugated secondary antibody and the appropriate color-developing reagents (Promega).

Raf-1 kinase assay. To perform the Syntide assay (10, 18), anti-Raf-1 monoclonal antibody or preimmune mouse (control) serum was added to oocyte or egg lysates (1 μg of antibody per 500 μl of lysate), and the mixture was rocked overnight at 4°C; 25 μl of resuspended protein A-Sepharose beads was added to each lysate, and the mixture was rocked for 1 h. The beads were washed with 0.5 M LiCl and water, resuspended in 38 μl of kinase buffer (25 mM Tris-HCl [pH 7.5], 10 mM $MnCl_2$, 10 μM ATP, 1 mM dithiothreitol, 25 mM β-glycerophosphate), 1 μl of 4 mM Syntide II peptide (GIBCO BRL), and 1 μl of [γ-^{32}P]ATP (6,000 Ci/mmol; Amersham), and incubated for 20 min at room temperature. The kinase reaction mix was spotted onto Whatman P81 paper and air dried. The paper was extensively washed with 0.85% phosphoric acid and then counted in scintillation fluid. Control immunoprecipitate counts were subtracted from Raf-1 immunoprecipitate counts.

MAP kinase assay. Anti-MAP kinase monoclonal antibody (or preimmune mouse immunoglobulin G for mock immunoprecipitation) and protein A-Sepharose beads were added to 500 μl of oocyte lysate, and the mixture was rocked for 3 h at 4°C. Immunoprecipitates were washed twice in 1 M NaCl and once in 25 mM HEPES (pH 7.5). The beads were resuspended in 36 μl of kinase buffer (25 mM HEPES [pH 7.5], 10 mM $MgCl_2$, 1 mM dithiothreitol, 50 μM ATP), 2 μl of [γ-^{32}P]ATP (6,000 Ci/mmol; Amersham), and 2 μl (1 mg/ml) of myelin basic protein (Sigma), and the kinase reaction mix was incubated at room temperature for 30 min. The beads were spun, and the supernatant was spotted onto P81 paper. The paper was washed in 0.85% phosphoric acid and evaluated by scintigraphy.

Histone H1 kinase assay. The histone H1 kinase assay was performed as follows. Ten microliters of clarified lysate was added to 35 μl of kinase buffer (20 mM HEPES [pH 7.4], 1 mM dithiothreitol, 10 mM $MgCl_2$, 100 μM ATP), 3 μl (1 mg/ml) of histone H1 (Sigma), and 2 μl of [γ-^{32}P]ATP (6,000 Ci/mmol; Amersham). This kinase reaction mix was incubated for 20 min at room temperature. Sample buffer was added, and each sample was run on an SDS–12% polyacrylamide gel. The gel was dried and evaluated by autoradiography.

RESULTS

Endogenous Raf-1 protein is phosphorylated following progesterone stimulation of immature oocytes. We first examined whether Raf-1 phosphorylation is increased during oocyte maturation. Lysates were made from oocytes at several intervals following the addition of progesterone. These lysates were examined for a change in the mobility of Raf-1 by Western blotting analysis using a monoclonal anti-human Raf-1 antibody (Fig. 1A). In untreated immature oocytes, the mobility of Raf-1 was characteristic of its unphosphorylated state. Several hours after progesterone stimulation, Raf-1 mobility was largely retarded. The level of Raf-1 protein appeared constant for the first few hours following progesterone addition; however, an approximately twofold increase in Raf-1 protein was noted following GVBD. MAP kinase mobility shift coincided with Raf-1 mobility shift following progesterone stimulation (Fig. 1B). Raf-1 from egg lysates treated with potato acid phosphatase had a faster mobility than did Raf-1 from untreated egg lysates, demonstrating that the shift in Raf-1 mobility is due to phosphorylation (data not shown).

Endogenous Raf-1 and MAP kinase activities increase following progesterone stimulation of immature oocytes. We next examined whether the Raf-1 mobility shift in oocytes correlated with Raf-1 activity. Raf-1 was immunoprecipitated from lysates of control and progesterone-treated oocytes, and an in vitro kinase assay was performed with the Syntide II peptide as a substrate. The kinase activity was increased significantly in the hyperphosphorylated (retarded mobility) form of Raf-1 found in progesterone-treated oocytes (Fig. 1C). To analyze whether the MAP kinase mobility shift in oocytes was indicative of increased activity, we tested the ability of MAP kinase immunoprecipitates to phosphorylate myelin basic protein in vitro. The kinase activity was substantially increased in the hyperphosphorylated (retarded mobility) form of MAP kinase found in progesterone-treated oocytes (Fig. 2A).

Introduction of an oncogenic form of Raf-1 into immature oocytes results in MAP kinase phosphorylation and meiotic maturation. Given that Raf-1 activity increased in parallel with MAP kinase activity during oocyte maturation, we considered the possibility that Raf-1 activity is upstream of MAP kinase activity. When 10 ng of RNA encoding an oncogenic mutant of Raf-1, v-Raf, was injected into oocytes, MAP kinase mobility was retarded (Fig. 2B). We further

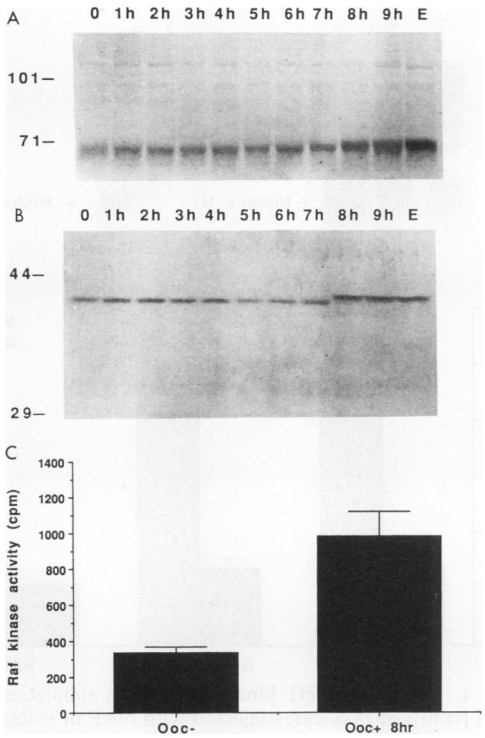

FIG. 1. (A) Shift in Raf-1 mobility during progesterone-induced oocyte maturation. Western blot analysis of oocyte lysates obtained at several intervals following progesterone stimulation and from unfertilized egg lysate (E) is shown. Approximately 50% of oocytes exhibited GVBD 7 h after progesterone stimulation. The anti-Raf-1 monoclonal antibody was used at a dilution of 1:500. (B) Shift in MAP kinase mobility during progesterone-induced oocyte maturation. Shown is a Western blot of the lower portion of the gel used in panel A. The commercial monoclonal anti-human MAP kinase antibody was used at a dilution of 1:1,000. Sizes in panels A and B are indicated in kilodaltons. (C) Increase in Raf-1 kinase activity during progesterone-induced oocyte (Ooc) maturation. Raf-1 immunoprecipitates from oocyte lysates were analyzed in an in vitro kinase assay using the Syntide II peptide as a substrate.

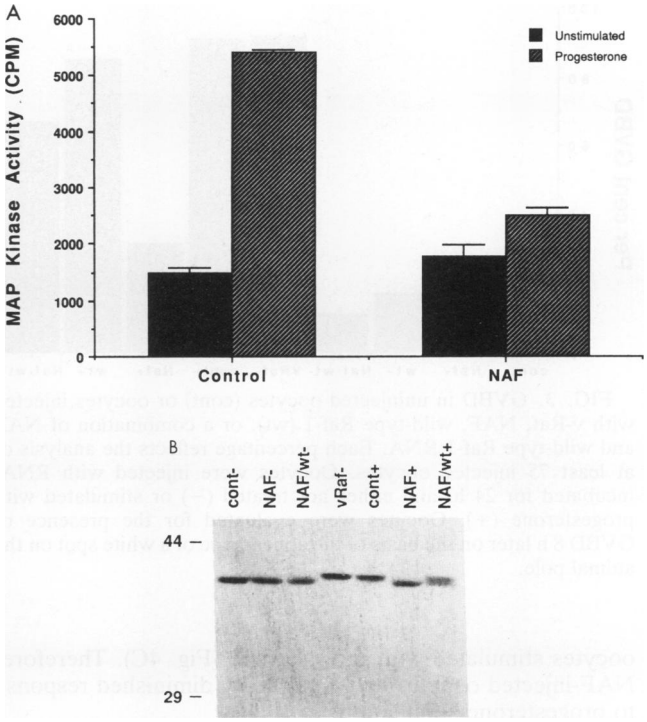

FIG. 2. (A) MAP kinase activity in uninjected and NAF RNA-injected oocytes. Oocytes were injected as for Fig. 1, and some were stimulated with progesterone for 8 h before lysates were made. Lysates were assayed for MAP kinase activity. Each column represents the average ± standard error of duplicate assays. (B) MAP kinase mobility shift in uninjected oocytes (control [Cont]) or oocytes injected with v-Raf, NAF, wild-type Raf-1 RNA (wt), or a combination of NAF and wild-type Raf-1 RNA. Oocytes were injected with RNA, incubated for 24 h, and either not treated (−) or stimulated with progesterone at 2 mg/ml (+). Protein encoded by the fusion of each RNA with the KT3 sequence was detected by Western blot analysis of oocyte lysates by using monoclonal antibodies (not shown). Sizes are indicated in kilodaltons.

hypothesized that Raf-1 activity could induce oocyte maturation. When v-Raf RNA was injected, GVBD was observed in over 90% of oocytes in the absence of progesterone (Fig. 3). Compared with uninjected control oocytes or oocytes injected with wild-type Raf-1 RNA, this increase in GVBD was statistically significant by chi-square analysis with continuity correction ($P = 0.0001$). Histone H1 kinase activity was increased approximately eightfold 12 h after the injection of v-Raf RNA in the absence of progesterone stimulation as measured in vitro (Fig. 4A).

We next tested whether wild-type Raf-1 RNA could induce oocyte maturation. When 10 ng of RNA encoding wild-type Raf-1 was injected into each oocyte, no shift in MAP kinase mobility was observed (data not shown), and the increase in GVBD was very slight compared with that of uninjected oocytes (Fig. 3). When a high dose (50 ng per oocyte) of RNA encoding wild-type Raf-1 was injected, GVBD was observed in a majority of oocytes (63%), and histone H1 kinase activation was noted (Fig. 4B).

Introduction of a dominant-negative Raf-1 mutant into oocytes blocks progesterone-induced meiotic maturation. We next considered the possibility that a dominant-negative version of Raf-1, termed NAF (not a functional Raf-1), could inhibit progesterone-induced MAP kinase activation and oocyte maturation. The NAF construct encodes a mutant Raf-1 that has a single amino acid substitution at the conserved ATP binding site. A similar mutant has been demonstrated to be a dominant-negative form of Raf-1 that blocks growth factor-induced mitogenesis in mammalian cell lines (9). The NAF mutant appears to function by specifically blocking activation of endogenous Raf-1 protein in *Xenopus* oocytes but does not directly block other kinases (15).

When oocytes were injected with 10 ng of NAF-encoding RNA and incubated for 24 h, NAF protein was easily detected by Western blotting with either an anti-KT3 or anti-Raf antibody. NAF has an apparent molecular mass 4 to 5 kDa greater than that of endogenous Raf-1. Approximately two- to threefold more NAF protein than endogenous Raf-1 protein was detected in oocytes injected with 10 ng of RNA (data not shown).

When progesterone was added to oocytes previously injected with 10 ng of RNA encoding NAF, MAP kinase mobility shift and activation did not occur (Fig. 2). Also, GVBD was less in NAF-injected oocytes following progesterone stimulation than in uninjected oocytes or oocytes injected with wild-type Raf-1 RNA (Fig. 3). This reduction in GVBD was significant by chi-square analysis ($P = 0.0001$). Histone H1 kinase activation was blocked in NAF-injected

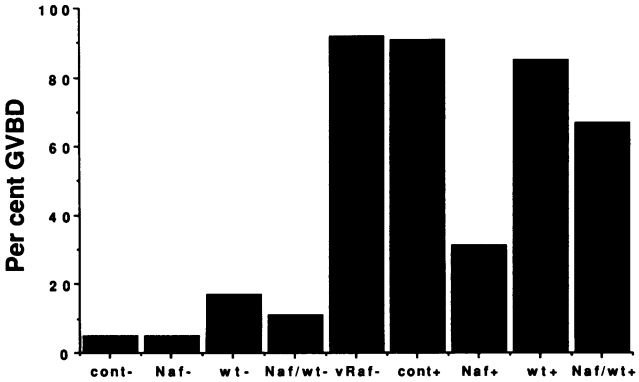

FIG. 3. GVBD in uninjected oocytes (cont) or oocytes injected with v-Raf, NAF, wild-type Raf-1 (wt), or a combination of NAF and wild-type Raf-1 RNA. Each percentage reflects the analysis of at least 75 injected oocytes. Oocytes were injected with RNA, incubated for 24 h, and either not treated (−) or stimulated with progesterone (+). Oocytes were evaluated for the presence of GVBD 8 h later on the basis of the appearance of a white spot on the animal pole.

oocytes stimulated with progesterone (Fig. 4C). Therefore, NAF-injected oocytes had a markedly diminished response to progesterone stimulation.

When NAF and wild-type Raf-1 are cointroduced into oocytes, response to progesterone is rescued. When progesterone was added to oocytes previously injected with equimolar concentrations of NAF and wild-type Raf-1 RNA, MAP kinase mobility was shifted (Fig. 2B), GVBD occurred at nearly the same rate as in uninjected oocytes (Fig. 3), and histone H1 kinase activity increased (Fig. 4C), indicating that wild-type Raf-1 overcame the effect of NAF. These results suggest that the effect of NAF on GVBD is specifically due to the inhibition of Raf-1 kinase activity.

Mos^{xe} activity is upstream of Raf-1 phosphorylation. Other investigators have shown that when mos^{xe} RNA or protein is injected into oocytes, GVBD and MPF activation occur within a few hours (5, 24, 28). We injected mos^{xe} RNA into immature oocytes in the absence of progesterone and obtained lysates at several time points. Raf-1 and MAP kinase exhibited mobility shifts characteristic of the activated states several hours after RNA injection (Fig. 5).

It is possible to block progesterone-induced oocyte maturation by injecting antisense oligonucleotides directed against mos^{xe} mRNA (24). We injected oocytes with such an antisense mos^{xe} oligonucleotide and added progesterone. Under the conditions used, antisense mos^{xe} oligonucleotides markedly reduced progesterone-induced GVBD. After 8 h of progesterone stimulation, GVBD was observed in 19% (12 of 62) of oocytes injected with antisense mos^{xe} oligonucleotides, compared with 91% (64 of 70) of control oocytes. Western blot analysis revealed that the antisense mos^{xe} oligonucleotides blocked progesterone-induced Raf-1 and MAP kinase mobility shifts (Fig. 5).

We also examined whether NAF could block GVBD induced by mos^{xe} RNA injection. We first injected oocytes with NAF or wild-type Raf-1 RNA. The next day, oocytes were injected a second time with mos^{xe} RNA. Oocytes injected with NAF and mos^{xe} exhibited significantly less GVBD than did oocytes injected with wild-type Raf-1 and mos^{xe} or with mos^{xe} alone (Fig. 6). This reduction in GVBD was significant by chi-square analysis ($P = 0.0001$).

We next examined whether v-Raf RNA could induce

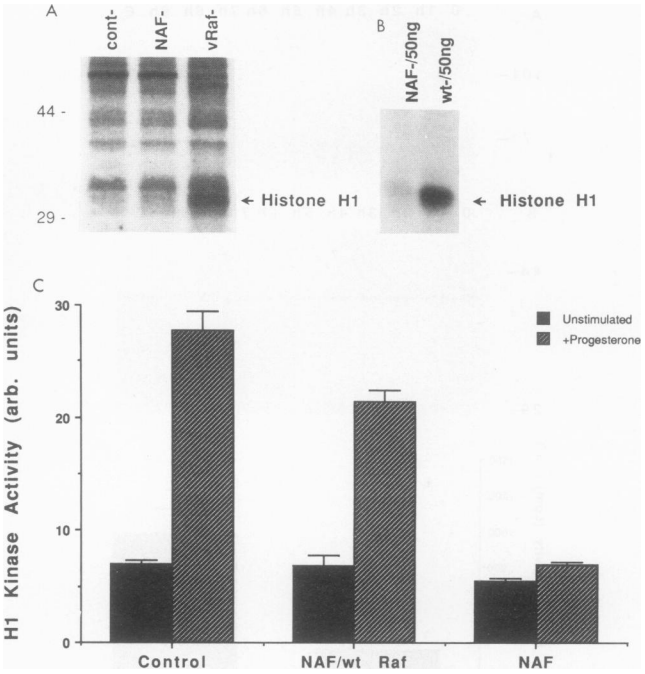

FIG. 4. (A) Histone H1 kinase activity in uninjected oocytes (control [cont]) or in oocytes injected with NAF or v-Raf RNA (10 ng per oocyte). Injected oocytes were incubated for 12 h at 18°C. Oocyte lysates were used in a histone H1 assay as described in the text. Sizes are indicated in kilodaltons. (B) Histone H1 kinase activity in oocytes injected with high-dose NAF RNA (50 ng per oocyte) or high-dose wild-type Raf-1 RNA (wt; 50 ng per oocyte). Injected oocytes were incubated for 12 h at 18°C. Oocyte lysates were used in a histone H1 assay as described in the text. (C) Histone H1 kinase activity in uninjected oocytes or in oocytes injected with NAF RNA alone (10 ng per oocyte) or with an equimolar mixture of NAF and wild-type Raf-1 RNA (wt; 20 ng per oocyte). Injected oocytes were incubated for 24 h at 18°C, and some were stimulated for 8 h with progesterone. Oocyte lysates were used in a histone H1 assay as described in the text, and the histone band was analyzed by densitometry. Each column represents the average ± standard error of triplicate determinations.

oocyte maturation in the absence of mos protein. Oocytes coinjected with v-Raf RNA and mos^{xe} oligonucleotides exhibited a shift in MAP kinase mobility (data not shown), a high rate of GVBD (Fig. 7A), and prominent histone H1 kinase activation (Fig. 7B).

DISCUSSION

The findings described above suggest that Raf-1 activation is an important regulatory step in *Xenopus* oocyte maturation and in MAP kinase activation. When immature oocytes are stimulated with progesterone, endogenous Raf-1 is phosphorylated and activated after several hours, concurrent with MAP kinase activation. Introduction of oncogenic Raf-1 RNA into immature oocytes results in GVBD, maturation promoting factor (MPF) activation, and MAP kinase phosphorylation. Introduction of dominant-negative Raf-1 (NAF) RNA into immature oocytes results in blockade of progesterone-induced GVBD, MPF activation, and MAP kinase activation. Cointroduction of wild-type and dominant-negative Raf-1 RNA into oocytes results in the rescue of progesterone responsiveness, implying that the effects of NAF are specific to Raf-1 inhibition.

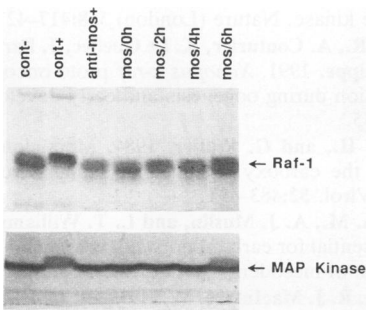

FIG. 5. Raf-1 and MAP kinase mobilities in oocytes injected with mos^{xe} antisense oligonucleotides (20 ng per oocyte) or with full-length mos^{xe} sense RNA (10 ng per oocyte). Oocytes injected with antisense mos^{xe} oligonucleotides and control oocytes were stimulated with progesterone for 8 h (+). Oocytes injected with full-length sense mos^{xe} RNA were not stimulated, and lysates were made at several intervals. In oocytes injected with full-length mos^{xe}, GVBD was observed in 50% of oocytes at 6 h. Oocyte lysates were analyzed by Western blotting as described in the text. The upper and lower panels represent different portions of the same gel. cont−, unstimulated control; cont+, stimulated control.

The results of these experiments strengthen observations made in vitro and with cultured mammalian cells that Raf-1 can activate MAP kinase (2, 6, 12). A precise definition of the mechanism by which Raf-1 activates MAP kinase in oocytes cannot be determined from these results. Intermediary molecules, such as the recently identified MAP kinase activator, may be involved in the Raf-1-induced MAP kinase activation in oocytes. Furthermore, the immediate downstream effects of MAP kinase activation in oocytes remain uncertain. Raf-1 kinase activity may not be limited to the activation of MAP kinase but may also be important in the activation of another enzyme that is critical for cell cycle progression.

When oocytes are stimulated by progesterone, the mos^{xe} protein is synthesized and activated (23). Recently, oocyte injection experiments demonstrated that mos^{xe} protein is necessary for the initiation of oocyte maturation and that mos^{xe} injection leads to MPF activation in the absence of protein synthesis (28). We hypothesized that Raf-1 and

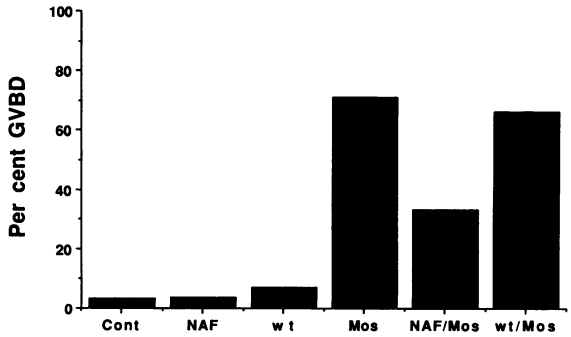

FIG. 6. GVBD in uninjected oocytes (cont) or in oocytes injected with mos^{xe} RNA alone, mos^{xe} and NAF RNA, or mos^{xe} and wild-type Raf-1 RNA (wt). Each percentage reflects the analysis of at least 150 injected oocytes. Oocytes were initially injected with water, NAF RNA (10 ng), or wild-type Raf-1 RNA (10 ng); 24 h later, oocytes were injected with mos^{xe} RNA (10 ng). Oocytes were observed for 8 h and were scored for the appearance of a white spot on the animal pole.

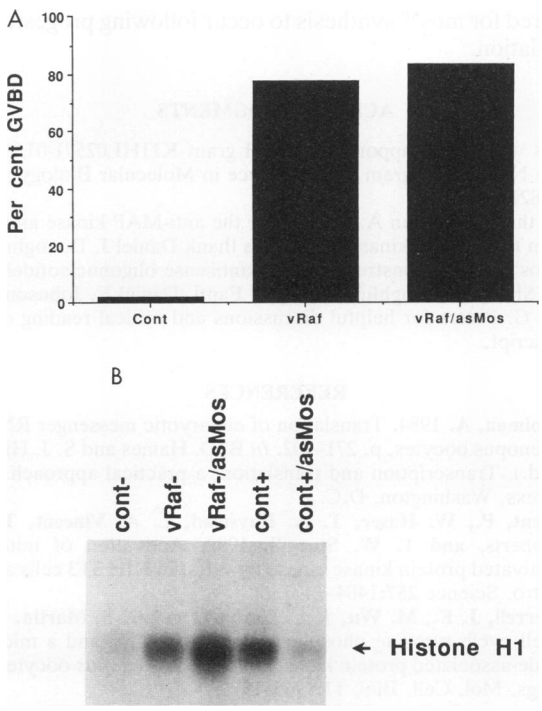

FIG. 7. (A) GVBD in uninjected oocytes (Cont) or in oocytes injected with v-Raf RNA alone or with a mixture of v-Raf RNA (10 ng per oocyte) and antisense mos^{xe} (AsMos) oligonucleotides (20 ng per oocyte). Each percentage reflects the analysis of at least 75 injected oocytes. Oocytes were injected with RNA as described for Fig. 6, incubated for 12 h, and then scored for the appearance of a white spot on the animal pole. (B) Histone H1 kinase activity in uninjected oocytes (cont) or in oocytes injected with v-Raf RNA alone (10 ng per oocyte), with a mixture of v-Raf RNA (10 ng per oocyte) and antisense mos^{xe} (AsMos) oligonucleotides (20 ng per oocyte), or with antisense mos^{xe} oligonucleotides alone (20 ng per oocyte). Oocytes were incubated in the presence (+) or absence (−) of progesterone (2 μg/ml) for 12 h at 18°C. Oocyte lysates were used in a histone H1 assay as described in the text.

mos^{xe} kinase activities were in the same signal transduction pathway. In this study, when mos^{xe} RNA was injected into oocytes, a Raf-1 mobility shift was observed. When antisense mos^{xe} oligonucleotides were injected into oocytes, progesterone-induced Raf-1 mobility shift was blocked. When oocytes were preinjected with NAF RNA, mos^{xe}-induced GVBD was blocked. Finally, oocytes coinjected with antisense mos^{xe} oligonucleotides and v-Raf RNA exhibited a high rate of GVBD and histone H1 kinase activation. These findings suggest that mos^{xe} activity is upstream of Raf-1 activity in progesterone-induced oocyte maturation. Also, there is now evidence in mos-transformed somatic cells that Raf-1 activity is downstream of mos (8).

An important question is whether mos^{xe} directly activates Raf-1 or whether it activates intermediary kinases which, in turn, activate Raf-1. Experiments are ongoing in our laboratory to determine the identity of the activator of Raf-1 in oocytes. The timing of Raf-1 activation in oocytes following progesterone stimulation is considerably delayed in comparison with growth factor-stimulated somatic cells. For example, when platelet-derived growth factor is added to cultured cells, Raf-1 activation occurs within 5 min (18). The delay in Raf-1 activation in oocytes may be a result of the time

required for mos^{xe} synthesis to occur following progesterone stimulation.

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