Inhibition of *mos*-induced Oocyte Maturation by Protein Kinase A

Ira Daar, Nelson Yew, and George F. Vande Woude

ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, P.O. Box B, Frederick, Maryland 21702

Abstract. The relationship between the *mos* protooncogene protein and cAMP-dependent protein kinase (PKA) during the maturation of *Xenopus* oocytes was investigated. Microinjection of the PKA catalytic subunit (PKA_c) into *Xenopus* oocytes inhibited oocyte maturation induced by the *mos* product but did not markedly affect the autophosphorylation activity of injected *mos* protein. By contrast, PKA_c did not inhibit maturation promoting factor (MPF) activation or germinal vesicle breakdown (GVBD) that was initiated by injecting crude MPF preparations. In addition, inhibiting endogenous PKA activity by microinjecting the PKA regulatory subunit (PKA_r) induced oocyte maturation that was dependent upon the presence of the endogenous *mos* product. Moreover, PKA_r potentiated *mos* protein–induced MPF activation in the absence of progesterone and protein synthesis. These data are consistent with the hypothesis that progesterone-induced release from G_2/M is regulated via PKA_c and that PKA_c negatively regulates a downstream target that is positively regulated by *mos*.

FULLY grown *Xenopus* oocytes are arrested in prophase of meiosis I and are induced to mature upon exposure to progesterone (29). Synthesis of the *mos* protooncogene product, $pp39^{mos}$, is required for the activation of maturation-promoting factor (MPF)[1], an activity responsible for coordinating the biochemical events of meiosis I and II (9, 22, 39, 41). Injecting the *mos* product into a two-cell embryo arrests the injected cell at metaphase, which led to the identification of *mos* as an active component of cytostatic factor (CSF) (41). CSF is a calcium-sensitive activity believed to be responsible for the arrest of an unfertilized egg at metaphase of meiosis II and for the stabilization of MPF (29, 31, 32, 41).

The observation that *mos* is synthesized prior to MPF activation during meiosis I, led us to propose that *mos* is an "initiator" and is required to activate MPF from pre-MPF (40). Furthermore, $pp39^{mos}$ is required at all stages during oocyte maturation (9, 22). *mos* RNA or protein can initiate MPF activation when microinjected into fully grown oocytes (16, 40, 50). Recently, using recombinant *mos* protein (MBP-mos^{xe}), we have shown that the protooncogene product is both necessary and sufficient to initiate meiosis I (50). However, injected oocytes do not progress to meiosis II in the absence of protein synthesis (50), even though *mos* is required during this period (9, 22). This suggests that additional proteins synthesized de novo are required for meiosis II and CSF arrest (50).

Progesterone markedly enhances MBP-mos^{xe}–induced germ vesicle breakdown (GVBD) in the absence of protein synthesis (50), suggesting that the hormone removes a biochemical block to MPF activation that pre-exists in the oocyte. This system provided a means for testing the biochemical events involved in oocyte maturation in the absence of protein synthesis requirements. cAMP-dependent protein kinase (PKA) has been implicated as a negative regulator of G_2/M transition and it is generally believed that progesterone stimulation of oocytes causes a transient decrease in cAMP levels. This, in turn, leads to a decrease in PKA activity and results in the dephosphorylation of a presumptive maturation-inhibiting phosphoprotein (44). While the role of cAMP and PKA in oocyte maturation is unclear (44) the following observations support the above hypothesis: progesterone inhibits adenylate cyclase activity in frog oocytes (13, 20, 34, 36); cAMP levels decrease during oocyte maturation in several organisms including frog (24, 25, 42), starfish (30), and mammals (43); the injection of phosphodiesterase induces maturation (7; Foerder, C. A., T. J. Martins, J. A. Beavo, and E. G. Krebs. 1982. *J. Cell Biol.* 95:304a), while inhibitors of phosphodiesterase prevent maturation (7, 37, 43); activators of adenylate cyclase cause an increase in cAMP levels, resulting in inhibition of *Xenopus* and mammalian oocyte maturation (17, 34, 49); and the injection of either the PKA regulatory subunit (PKA_r), which binds to and inactivates the catalytic subunit, or the PKA inhibitory peptide (PKI) induces maturation (26), while the catalytic subunit of PKA (PKA_c) inhibits oocyte maturation in amphibians (26) and mammals (7).

Since progesterone influences the PKA pathway and

Dr. Daar's present address is Biological Response Modifiers Program, NCI-Frederick Cancer Research and Development Center, P.O. Box B, Frederick, MD 21702.

1. *Abbreviations used in this paper*: CSF, cytostatic factor; GVBD, germ vesicle breakdown; MBS, modified Barth solution; MPF, maturation promoting factor; PKA, protein kinase; PKI, PKA inhibitory peptide.

potentiates *mos*-induced MPF activation in the absence of protein synthesis, we examined the effects of the PKA subunits on *mos* function. We show that the PKA_c does not affect MPF-induced maturation, but markedly inhibits MBP-*mos*^{xe}–induced GVBD. By contrast, in the absence of protein synthesis the regulatory subunit (PKA_r) potentiates MPB-*mos*^{xe} promotion of GVBD almost as efficiently as progesterone.

Materials and Methods

Frogs and Oocytes

Xenopus laevis females were purchased from *Xenopus* I (Ann Arbor, MI). Oocytes were surgically removed and defolliculated by incubation in modified Barth solution (MBS; 88 mM NaCl, 1 mM KCl, 2.5 mM $NaHCO_3$, 10 mM Hepes, pH 7.5, 0.82 mM $MgSO_4$, 0.33 mM $Ca[NO_3]_2$, 0.41 mM $CaCl_2$) containing collagenase A (1.5 mg/ml; Boehringer Mannheim Biochemicals, Indianapolis, IN) for 2 h (18). After several washes, oocytes were cultured overnight in 50% Leibovitz-15 media (Gibco Laboratories, Grand Island, NY).

Injections

18 h after oocytes isolation, microinjections were performed by using an Attocyte injector (ATTO Instruments, Rockville, MD) with 30 nl of one or several of the following: MBP-*mos*^{xe} protein produced in *Escherichia coli* and isolated as described (50), protein kinase A catalytic subunit (Promega, Madison, Wisc.), PKA_c RNA transcripts synthesized in vitro as described (10), protein kinase A regulatory subunit (Promega, Madison, WI), and *mos* specific oligonucleotides (39) (60 ng/oocyte). The H-Ras^{lys12} p21 protein was produced in and purified from *E. coli* (a gift of Angel Nebreda, National Institute of Allergy and Infectious Diseases). Oocytes were scored for GVBD by the appearance of a white spot at the animal pole as well as by manual dissection after fixation in 10% TCA.

Immunoprecipitations and In Vitro Kinase Assays

Groups of 10 oocytes were homogenized in lysis buffer (150 mM NaCl, 10 mM sodium phosphate, pH 7.2, 1 mM EDTA, 0.1% NP-40) containing 2 mM DMSF and clarified by centrifugation at 14,000 *g* for 5 min at 4°C. To the supernatant, 5S mAb was added, incubated 1 h, and complexed with 10% protein A Sepharose in lysis buffer. The pellet was washed three times with lysis buffer, then resuspended in 50 μl of kinase reaction buffer (150 mM NaCl, 10 mM Hepes, pH 7.2, 15 mM $MnCl_2$, 2 mM DTT, 10 μM ATP) and incubated in the presence of 20 μCi of [γ-^{32}P]ATP (New England Nuclear, Boston, MA) for 15 min at room temperature. The pellets were washed twice with lysis buffer and resuspended in 2× sample buffer, analyzed by SDS-PAGE buffer on a 12% gel, and then visualized by autoradiography.

Ribosomal Subunit S6 Phosphorylation

Stage VI oocytes were prelabeled for 3.5 h at 20°C in MBS containing $^{32}P_i$ (0.3 mCi/ml; Amersham Corp., Arlington Heights, IL). Oocytes were injected with either MBP-*mos*^{xe} protein alone (9 ng per oocyte) or MPB-*mos*^{xe} (9 ng) with the PKA_c (42 ng per oocyte; Promega Biotec, Madison WI) and incubated in fresh MBS for 9 h at 20°C. Over this period, groups of 20 oocytes were harvested and homogenized in 1 ml of ribosome isolation buffer (50 mM Pipes, pH 7.5, 5 mM $MgCl_2$, 5 mM KCl, 50 mM NaF, 4 μM EDTA, 1% deoxycholate, 1% Triton X-100). Extracts were clarified by centrifugation at 14,000 *g* for 16 h at 4°C. The ribosome pellets were suspended in 2× sample buffer, analyzed by SDS-PAGE on a 12% gel, and visualized by autoradiography. The ribosomal S6 subunit bands were excised and counted.

Histone H1 Kinase Assays

Crude MPF extracts were prepared by homogenizing groups of 10 to 20 oocytes in 20 to 40 μl of extraction buffer (80 mM β-glycerophosphate, 20 mM EGTA, 15 mM $MgCl_2$, 20 mM Hepes, pH 7.2, 1 mM ATP, 1 mM DTT, and 5 mM NaF). Homogenates were clarified by centrifugation at 14,000 *g* for 5 min at 4°C. 2 μl of the supernatant were added to 50 μl of stabilization buffer (80 mM β-glycerophosphate, 20 mM EGTA, 50 mM $MgCl_2$, 2.5 mM PMSF, 10 μg of leupeptin per ml, 10 μM protein kinase A inhibitor). The histone H1 kinase assay was performed by adding 10 μl of stabilized extract to 6 μl of a mixture containing 2 μg of histone H1 (Sigma, St. Louis, MO), 1 mM ATP, and 1.5 μCi of [γ-^{32}P]ATP. The reaction was incubated 15 min at room temperature, and then stopped with an equal volume of 2× sample buffer. Samples were resolved by SDS-PAGE on a 10% gel, and then fixed and autoradiographed.

Results

Inhibition of mos-induced Oocyte Maturation by the PKA Catalytic Subunit

PKA_c injection into *Xenopus* oocytes blocks progesterone-induced meiotic maturation (26). We tested the influence of PKA activity on MBP-*mos*^{xe} induced maturation. MBP-*mos*^{xe} plus increasing units of PKA_c protein were coinjected into fully grown oocytes (Fig. 1). At 1 ng per oocyte, PKA_c prevented MBP-*mos*^{xe} induction of maturation in 50% of the oocytes (Fig. 1). Maximum inhibition was observed when 4 ng of PKA_c was injected per oocyte. Similar levels of PKA_c also inhibit progesterone induced GVBD (data not shown). When MBP-*mos*^{xe} was injected with PKA_c and PKA_r, GVBD occurred in 92% of oocytes, showing that the PKA_c inhibition was specifically abrogated by the regulatory subunit. PKA_c also prevented the appearance of MPF and its associated histone H1 kinase activity (Figs. 1 and 2). Moreover, the *ras* oncoprotein (ras^{val12}), which also efficiently induces GVBD (4, 8) and has been shown to be antagonistic to the PKA pathway (4, 38), was also blocked by injection of PKA_c (Fish, S., D. Grieco, V. E. Arredimento, and M. E. Gottesman, unpublished data) (Fig. 2). By contrast, PKA_c did not inhibit crude MPF preparations from efficiently inducing meiotic maturation (Fig. 2), even when PKA_c was injected 1 h before MPF injection (data not shown). These results show that PKA_c activity inhibits both *ras* or *mos* maturation either directly or indirectly by negatively regulating a downstream substrate. They also suggest that PKA_c acts as an early inhibitor of maturation, upstream from MPF.

Kinetics of PKA_c Inhibition during Maturation

The period of PKA_c sensitivity was determined in maturing oocytes. Oocytes were either treated with progesterone or in-

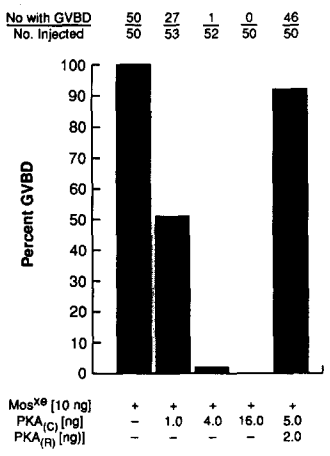

Figure 1. Sensitivity of MBP-*mos*^{xe} to inhibition of GVBD by the PKA catalytic subunit. Oocytes were injected with 10 ng of MBP-*mos*^{xe} along with 0 to 16 ng of PKA_c. GVBD was examined 12 h later. The ratio of the number of oocytes with GVBD to the total number injected is displayed above each bar. + denotes the presence of a reagent, while – indicates its absence.

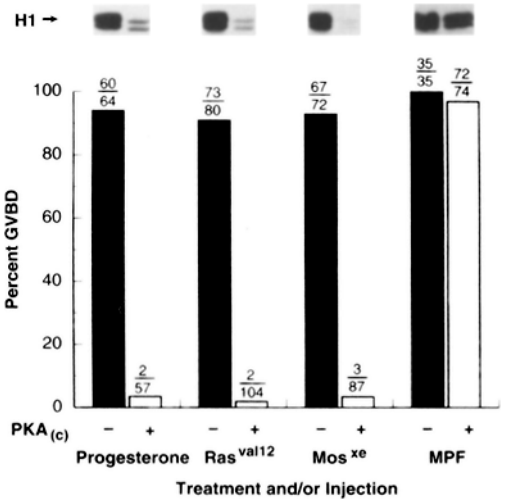

Figure 2. Inhibition of MBP-*mos*^{xe} and other inducers of GVBD by the PKA catalytic subunit. Oocytes were either treated or injected as indicated along with the coinjection of PKA_c (42 ng) (+) or in its absence (−). GVBD was examined 12–18 h later. The ratio of the number of oocytes with GVBD to the total number injected is displayed above each bar. Histone H1 kinase assays were performed on extracts from 10 appropriately injected oocytes and the autoradiograph is displayed above each bar.

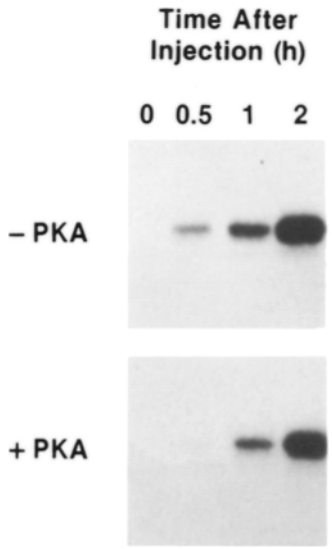

Figure 4. MBP-*mos*^{xe} activation in the presence of PKA. *Xenopus* oocytes were injected with MBP-*mos*^{xe} (9 ng per oocyte) with or without PKA_c (42 ng or 3 U per oocyte). At the indicated time, 10 oocytes were collected and lysed, and MBP-*mos*^{xe} was immunoprecipitated with a *Xenopus mos*-specific antibody. An in vitro kinase assay was then performed on the immune complex.

jected with MBP-*mos*^{xe} protein and followed by PKA_c injection at various times (Fig. 3 *A*). 50% of the oocytes resisted the inhibitory effect of PKA_c when the subunit was injected 2.5 to 3 h after progesterone treatment (Fig. 3, *A* and *B*). This period represents 0.56 to 0.67 $GVBD_{50}$ (a time which corresponds to MPF activation and protein synthesis independence). 50% of the MBP-*mos*^{xe}-injected oocytes became PKA_c resistant by 3.0 to 3.5 h after *mos* injection, or at 0.67 to 0.77 $GVBD_{50}$ (Fig. 3, *A* and *B*). The difference observed in the times of PKA_c resistance between the two groups may be the result of the more synchronous nature of progesterone exposure when compared to the time required for microinjection of MBP-*mos*^{xe} protein as well as the time required for this protein to be activated (50). These data are consistent with those previously reported (26), but suggest that the PKA inhibitory effect persists in meiosis I until maturation becomes protein synthesis independent at ~0.6 $GVBD_{50}$ (40).

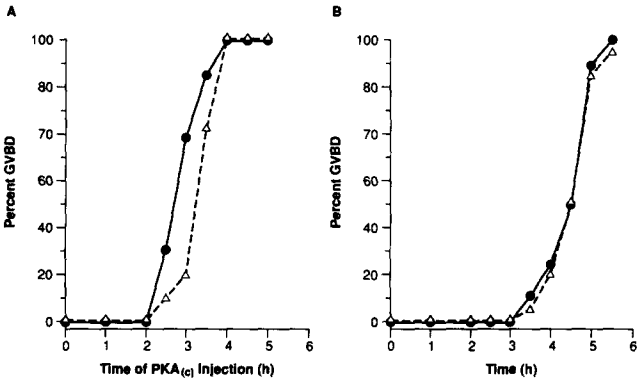

Figure 3. (*A*) Determination of the PKA_c-sensitive period. Oocytes were either exposed to progesterone (5 μg/ml) (●—●) or injected with MBP-*mos*^{xe} protein (10 ng) (△—△). At various times thereafter, 15 to 18 oocytes were injected with PKA_c (42 ng), and the percentage of oocytes that underwent GVBD was scored 7 h later. (*B*) Kinetics of GVBD induction by progesterone or MBP-*mos*^{xe} protein. MBP-*mos*^{xe} protein (9 ng) (△—△) was injected into each of 45 oocytes. Another 32 oocytes were exposed to progesterone (10 μg/ml) (●—●) at the same time, and the two sets of oocytes were scored for GVBD as a function of time.

*PKA_c Does Not Inhibit MBP-*mos^{xe} *Activation In Vivo*

To determine whether PKA_c inhibition of maturation results from inhibition of *mos* activity, we injected MBP-*mos*^{xe} either alone or with the PKA_c subunit into fully grown oocytes. We performed *mos* immune complex kinase assays on extracts prepared at various times after injection and measured MBP-*mos*^{xe} autophosphorylation activity (50). These results show that MBP-*mos*^{xe} autophosphorylation was not blocked by PKA_c and there was only a slight delay in the appearance of maximal activity (~0.5 h) (Fig. 4). Moreover, phosphorylation of MBP-*mos*^{xe} by PKA_c in vitro did not diminish its autophosphorylation activity (data not shown) even though PKA_c prevents GVBD induced by MBP-*mos*^{xe} protein (Figs. 1 and 2). These results suggest that the target of PKA_c inhibition may not be the *mos* product but, rather, a substrate downstream in the meiotic initiation pathway, prior to GVBD.

Inhibition of S6 Phosphorylation by PKA_c

The ribosomal subunit S6 is phosphorylated during oocyte maturation induced by insulin or progesterone (33) and requires endogenous *mos* protein function (3). We determined whether PKA_c has an effect on MBP-*mos*^{xe}–induced S6 phosphorylation. Oocytes were pre-labeled with $^{32}P_i$ for 3.5 h and subsequently injected with MBP-*mos*^{xe} protein either alone or with the PKA_c protein. Over a period of 9 h, ribosomes were isolated and phosphoproteins were analyzed by SDS-PAGE. Again, the injection of PKA_c inhibited MBP-*mos*^{xe}–induced GVBD (Fig. 5) and S6 protein phos-

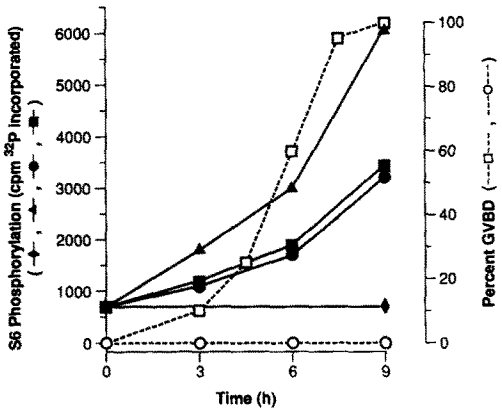

Figure 5. Phosphorylation of S6 protein in oocytes injected with MBP-*mos*^{xe} and PKA catalytic subunit. One hundred ^{32}P-labeled stage VI oocytes were injected with MBP-*mos*^{xe} alone (▲), or MBP-*mos*^{xe} and PKA catalytic subunit (●), PKA catalytic subunit alone (■), or buffer (♦). 20 injected oocytes were harvested at the indicated times after injection, and phosphorylated S6 proteins in ribosomes were analyzed by SDS-PAGE followed by autoradiography. Maturation of oocytes induced by MBP-*mos*^{xe} alone (□) and with PKA catalytic subunit (○) was determined for the same set of oocytes.

phorylation was ~50% lower than in oocytes injected with MBP-*mos*^{xe} alone or PKA$_c$ alone (Fig. 5).

The PKA Regulatory Subunit Enhances MBP-mos^{xe}-induced Maturation

Progesterone potentiates the ability of MBP-*mos*^{xe} to induce GVBD in the presence of cycloheximide. Since progesterone treatment of oocytes leads to reduced cAMP levels and presumably reduced PKA activity, we determined whether PKA$_r$ enhances MBP-*mos*^{xe}-induced maturation in the absence of protein synthesis and progesterone. The concentrations of MBP-*mos*^{xe} or PKA$_r$ were titrated for their ability to induce GVBD in cycloheximide-treated oocytes. The injection of 2.0 ng of MBP-*mos*^{xe} into cycloheximide-treated oocytes failed to cause GVBD (Fig. 6, bar *2*). 1–3 U of PKA$_r$-induced GVBD (26) in 60 to 88% of untreated oocytes, respectively, while PKA$_r$ did not cause GVBD in the presence of cycloheximide (Fig. 6, bars *7–9*) even with progesterone present (data not shown). As previously shown, progesterone potentiates the induction of GVBD by MBP-*mos*^{xe} in cycloheximide-treated oocytes (50) (Fig. 6, bar *3*). When levels of PKA$_r$ suboptimal for inducing maturation were injected with MBP-*mos*^{xe} in the presence of cycloheximide, a substantial increase in the percentage of oocytes undergoing GVBD was observed. Thus, from a baseline of 2% for MBP-*mos*^{xe} alone, 63, 81, and 88% GVBD was observed for 1.0, 2.0, and 3.0 U of PKA$_r$, respectively (Fig. 6, bars *4–6*). These data show that PKA$_r$ potentiates MBP-*mos*^{xe}–induced GVBD almost as effectively as progesterone in the absence of protein synthesis.

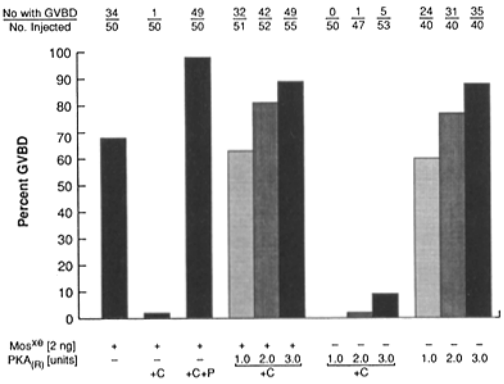

Figure 6. Synergy between MBP-*mos*^{xe} and the PKA regulatory subunit in the absence of protein synthesis. Between 20 and 80 oocytes were injected with the indicated amounts of MBP-*mos*^{xe} and/or PKA regulatory subunit and scored for GVBD 8 to 12 h later. *Mos*^{xe}, MBP-*mos*^{xe} protein; *PKA$_r$* regulatory subunit; *C*, cycloheximide (10 μg/ml); *P*, progesterone.

PKA$_r$-induced Maturation Requires pp39^{mos} Synthesis

To determine whether the synergy between MBP-*mos*^{xe} and the PKA regulatory subunit is due to PKA activity functioning downstream of *mos* or through a parallel (mutually dependent) pathway, we metabolically labeled oocytes with [^{35}S]methionine and microinjected PKA$_r$. After 4 h, the *mos* product was precipitated from oocytes using a *mos* specific antibody. Oocytes treated with either progesterone or injected with PKA$_r$ expressed *mos* product indicating that *mos* synthesis was induced, while untreated oocytes did not express pp39^{mos} (Fig. 7 *A*). We tested whether pp39^{mos} is required for PKA$_r$ induced oocyte maturation by blocking *mos* protein formation with antisense oligonucleotides (39). Only 3% of the antisense injected oocytes underwent GVBD, while 87% of control sense oligonucleotide injected oocytes matured with PKA$_r$ (Fig. 7 *B*). These data show that PKA and *mos* function through a mutually dependent pathway.

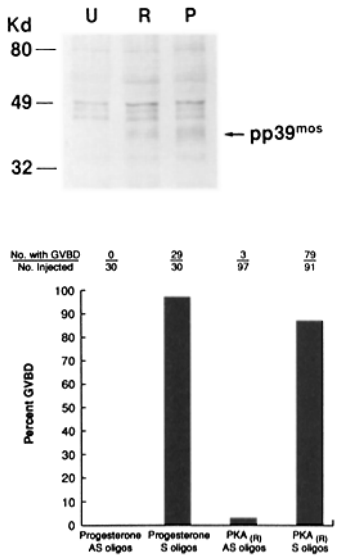

Figure 7. (*A*) The induction of pp39^{mos} synthesis in PKA$_r$-injected oocytes. 50 oocytes were metabolically labeled for 3 h in MBS containing ^{35}S-translabel (0.5 Mci/ml), and then either injected with PKA$_r$ (*R*) (3.0 U), or treated with progesterone (*P*) (10 μg/ml) or left untreated (*U*). After 4 h, the oocytes were subjected to immunoprecipitation with a *mos* specific antibody (5S) and analyzed by 10% SDS-PAGE. (*B*) The requirement for pp39^{mos} synthesis in PKA$_r$ injected oocytes. Fully grown oocytes were injected with either antisense *mos* oligonucleotides (39) (60 ng/oocyte) or sense oligonucleotides (60 ng/oocyte). After one hour, these oocytes were either treated with progesterone or injected with PKA$_r$ (3.0 U). GVBD was examined externally and internally after 12–14 h. The ratio of oocytes with GVBD to the number injected is displayed over each bar.

Discussion

In *Xenopus* oocytes, the introduction of 1–4 ng of PKA_c blocks MPF activation initiated by progesterone or the *mos* product. However, crude MPF preparations can induce GVBD (Fig. 2) and meiotic progression through meiosis II in the presence of PKA_c (data not shown). Since the MPF preparations are crude, they contain many other proteins in addition to the active cdc2–cyclin complex, and thus cannot be excluded from influencing this result. The above results suggested that PKA inhibits, while *mos* positively regulates, a downstream target that is upstream of MPF during meiosis I, but this target is either inactive or absent between meiosis I and II, a period requiring *mos* function (9, 22). Consistent with this idea, PKA_c prevents maturation initiated by the *ras* oncoprotein, which can induce GVBD when *mos* translation is blocked (8) and in the presence of cycloheximide (1).

The results also indicate a dual role for $pp39^{mos}$ as "initiator" and as CSF, since this inhibition only affects *mos* "initiator" function and not *mos* activity during the latter part of meiosis. The time period when MBP-mos^{xe}–induced GVBD becomes resistant to PKA activity (0.67–0.77 $GVBD_{50}$) in 50% of oocytes corresponds to the time when GVBD becomes independent of protein synthesis and MPF becomes activated. These results are not significantly different than those reported in an earlier study by Maller and Krebs (26), where 50% of GVBD was insensitive to PKA_c at 2.5 h (.75 $GVBD_{50}$). Consistent with these findings, it has recently been reported that when oocytes are treated with known elevators of intracellular cAMP (IBMX and cholera toxin), the nondegradable Δ90 cyclin cannot induce MPF activation, and in oocytes depleted of endogenous cyclins, $p34^{cdc2}$ kinase is inactivated by phosphorylation on tyrosine 15 (35). Although the detectable decrease in cAMP levels occurs early in progesterone stimulated oocytes, this does not rule out the possibility that the target of PKA activity is involved in the activation of the MPF complex or possibly a component of the MPF complex which is already present in an inactive form. Although PKA_c inhibited MBP-mos^{xe}–induced GVBD, it did not prevent the activation of the *E. coli*–expressed recombinant protein in vivo. It is possible that PKA activity inhibits the ability of $pp39^{mos}$ to phosphorylate an important substrate required for MPF activation, but this would have to be accomplished without suppressing its autokinase activity.

Ribosomal S6 protein phosphorylation is implicated in cell proliferation and transformation (5, 6, 46, 48). In *Xenopus* oocytes, the ribosomal subunit S6 is hyperphosphorylated after the initiation of meiotic maturation by progesterone (12, 33), insulin (11, 25), and several oncogene products including *ras* (3, 21), v-*src* (45), v-*abl* (28), and *tpr-met* (10). While hormonal stimulation of S6 phosphorylation is inhibited by *mos*-depletion (3), S6 kinase activity induced by activated *ras* or *tpr-met* appears to be only partially affected by the lack of *mos* product (3, 10). In this study, nearly 50% of the ribosomal subunit S6 phosphorylation induced by MBP-mos^{xe} was suppressed by PKA activity, however, the remaining 50% appears to be the result of the injected PKA_c. Thus, PKA_c inhibits S6 phosphorylation induced by *mos*.

The cAMP-dependent protein kinase has been implicated as a negative regulator of meiosis and early mitosis in several organisms. Recently, inhibition of PKA has also been reported to play a pivotal role in the G_2/M transition of mammalian fibroblasts (23). We have shown that MBP-mos^{xe}–induction of the G_2/M transition in cycloheximide-treated oocytes was potentiated by progesterone exposure, suggesting a possible role for the inhibition of PKA in this event (50). Here, we report a synergistic effect between PKA_r and MBP-mos^{xe} that enhanced the number of oocytes undergoing the G_2/M transition in the absence of protein synthesis, thereby mimicking the effect of progesterone. It has been previously shown that during oocyte maturation certain substrates are hyperphosphorylated, while other proteins are dephosphorylated (27), and it has been suggested that these dephosphorylation events may be the result of PKA inactivation (23). It is possible that *mos* and PKA_c are antagonists, where *mos* may inactivate an inhibitor of MPF activation, while PKA_c phosphorylates this inhibitor or an activator of this inhibitor, and thereby causes its activation. However, it is also possible that *mos* may activate, while PKA_c inactivates, an activator of pre-MPF. We have shown here that PKA_r requires *mos* synthesis to activate MPF. Perhaps, as has been suggested (19), progesterone acts by releasing the brakes (in this case, inhibiting PKA activity) and stepping on the accelerator (*mos* synthesis). Curiously, both PKA and $pp39^{mos}$, two proteins with significant homology (2), have been shown to associate with microtubules and phosphorylate tubulin in vitro (47, 51). Perhaps the antagonism between *mos* and PKA involves microtubule modification. Collectively, our results suggest that the inhibitory effect of PKA is a late step in the initiation of MPF activation and that inactivation of PKA, along with the promoting activities of the *mos* product, leads to MPF activation.

We thank A. Gian Ascione and Linda Miller for excellent technical assistance, Renping Zhou for helpful discussion, James Resnick and Deborah Morrison for critical reading of the manuscript, Anne Arthur for editorial assistance, and Joan Hopkins and Michelle Reed for exceptional performance in preparing the manuscript.

Research sponsored by the National Cancer Institute, DHHS, under contract no. NO1-CO-74101 with ABL. The contents of this publication do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Received for publication 18 September 1992 and in revised form 12 November 1992.

References

1. Allende, C. C., M. V. Hinrichs, E. Santos, and J. E. Allende. 1988. Oncogenic *ras* protein induces meiotic maturation of amphibian oocytes in the presence of protein synthesis inhibitors. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 234:426–430.
2. Barker, W. C., and M. O. Dayhoff. 1982. Viral src gene products are related to the catalytic chain of mammalian cAMP-dependent protein kinase. *Proc. Natl. Acad. Sci. USA.* 79:2836–2839.
3. Barrett, C. B., R. M. Schroetke, F. A. Van der Hoorn, S. K. Nordeen, and J. L. Maller. 1990. Ha-$ras^{val12thr59}$ activates S6 kinase and $p34^{cdc2}$ kinase in *Xenopus* oocytes: evidence for c-mos^{xe} dependent and independent pathways. *Mol. Cell. Biol.* 10:310–315.
4. Birchmeier, C., D. Broek, and M. Wigler. 1985. *ras* proteins can induce meiosis in *Xenopus* oocytes. *Cell.* 43:615–621.
5. Blenis, J., C. J. Kuo, and R. L. Erikson. 1987. Identification of a ribosomal protein S6 kinase regulated by transformation and growth promoting stimuli. *J. Biol. Chem.* 262:14373–14376.
6. Blenis, J., Y. Sugimoto, H. P. Blemann, and R. L. Erikson. 1985. Analysis of S6 phosphorylation in quiescent cells stimulated with serum growth factors, a tumor promoter, or by expression of the Rous sarcoma virus transforming gene product. *Cancer Cells.* 3:381–388.

7. Bornslaeger, E. A., P. Mattei, and R. M. Schultz. 1986. Involvement of cAMP-dependent protein kinase and protein phosphorylation in regulation of mouse oocyte maturation. *Dev. Biol.* 114:453–462.
8. Daar, I., A. R. Nebreda, N. Yew, P. Sass, R. Paules, E. Santos, M. Wigler, and G. F. Vande Woude. 1991. The *ras* oncoprotein and M-phase activity. *Science (Wash. DC).* 253:74–76.
9. Daar, I. O., R. S. Paules, and G. F. Vande Woude. 1991. A characterization of cytostatic factor activity from *Xenopus* eggs and c-*mos*-transformed cells. *J. Cell Biol.* 114:329–335.
10. Daar, I. O., G. A. White, S. M. Schuh, D. K. Ferris, and G. F. Vande Woude. 1991. *tpr-met* oncogene product induces maturation-promoting factor activation in *Xenopus* oocytes. *Mol. Cell. Biol.* 11:5985–5991.
11. El-Etr, M., S. Schorderet-Slatkine, and E. E. Baulieu. 1979. Meiotic maturation in *Xenopus laevis* oocytes initiated by insulin. *Science (Wash. DC).* 205:1397–1399.
12. Erikson, E., D. Stefanovic, J. Blenis, R. L. Erikson, and J. L. Maller. 1987. Antibodies to *Xenopus* egg S6 kinase II recognize S6 kinase from progesterone- and insulin-stimulated *Xenopus* oocytes and from proliferating chicken embryo fibroblasts. *Mol. Cell. Biol.* 7:3147–3155.
13. Finidori-Lepicard, J., S. Schorderet-Slatkine, J. Hanoune, and E. E. Aulieu. 1981. Progesterone inhibits membrane-bound adenylate cyclase in *Xenopus laevis* oocytes. *Nature (Lond.).* 292:255–257.
14. Deleted in proof.
15. Deleted in proof.
16. Freeman, R. S., K. M. Pickham, J. P. Kanki, B. A. Lee, S. V. Pena, and D. S. Donoghue. 1989. *Xenopus* homolog of the *mos* protooncogene transforms mammalian fibroblasts and induces maturation of *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA.* 86:5805–5809.
17. Goodhardt, M., N. Ferry, M. Buscaglia, E. E. Baulieu, and J. Hanoune. 1984. Does the guanine nucleotide regulatory protein Ni mediate progesterone inhibition of *Xenopus* oocyte adenylate cyclase? *EMBO (Eur. Mol. Biol. Organ.) J.* 3:2653–2657.
18. Gurdon, J. B., and M. P. Wickens. 1983. The use of *Xenopus* oocytes for the expression of cloned genes. *Methods Enzymol.* 101:370–387.
19. Hunt, T. 1992. Cell cycle arrest and c-*mos*. *Nature (Lond.).* 335:587–588.
20. Jordana, X., C. C. Allende, and J. E. Allende. 1981. Guanine nucleotides are required for progesterone inhibition of amphibian oocyte adenylate cyclase. *Biochem. Int.* 3:527–532.
21. Kamata, T., and H.-F. Kung. 1990. Modulation of maturation and ribosomal S6 phosphorylation in *Xenopus* oocytes by microinjection of oncogenic *ras* protein and protein kinase C. *Mol. Cell. Biol.* 10:880–886.
22. Kanki, J. P., and D. J. Donoghue. 1991. Progression from meiosis I to meiosis II in *Xenopus* oocytes requires *de novo* translation of the mos^{xe} protooncogene. *Proc. Natl. Acad. Sci. USA.* 88:5794–5798.
23. Lamb, J., J.-C. Cavadore, J.-C. Labbe, R. A. Maurer, and A. Fernandez. 1991. Inhibition of cAMP-dependent protein kinase plays a key role in the induction of mitosis and nuclear envelope breakdown in mammalian cells. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:1523–1533.
24. Maller, J. 1983. Interaction of steroids with the cyclic nucleotide system in amphibian oocytes. *Adv. Cyclic Nucleotide Res.* 15:295–236.
25. Maller, J. L. 1985. Regulation of amphibian oocyte maturation. *Cell Diff.* 16:211–221.
26. Maller, J. L., and E. G. Krebs. 1977. Progesterone-stimulated meiotic cell division in *Xenopus* oocytes. *J. Biol. Chem.* 252:1712–1718.
27. Maller, J. L., and D. S. Smith. 1985. Two-dimensional polyacrylamide gel analysis of changes in protein phosphorylation during maturation of *Xenopus* oocytes. *Dev. Biol.* 109:150–156.
28. Maller, J. L., J. G. Foulkes, E. Erikson, and D. Baltimore. 1985. Phosphorylation of ribosomal protein S6 on serine after microinjection of the Abelson murine leukemia virus tyrosine-specific protein kinase into *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA.* 82:272–276.
29. Masui, Y., and H. J. Clarke. 1979. Oocyte maturation. *Int. Rev. Cytol.* 57:185–282.
30. Meijer, L., W. Dostmann, H. H. Genieser, E. Butt, and B. Jastorff. 1989. Starfish oocyte maturation: evidence for a cyclic AMP-dependent inhibitory pathway. *Dev. Biol.* 133:58–66.
31. Murray, A. W., M. J. Solomon, and M. W. Kirschner. 1989. The role of cyclin synthesis and degradation in the control of maturation promoting factor. *Nature (Lond.).* 339:280–285.
32. Newport, J. W., and M. W. Kirschner. 1984. Regulation of the cell cycle during *Xenopus* development. *Cell.* 37:731–742.
33. Nielsen, P. J., G. Thomas, and J. L. Maller. 1982. Increased phosphorylation of ribosomal protein S6 during meiotic maturation of *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA.* 79:2937–2941.
34. Olate, J., C. C. Allende, J. E. Allende, R. D. Sekura, and L. Birnbaumer. 1984. Oocyte adenyl cyclase contains Ni, yet the guanine nucleotide-dependent inhibition by progesterone is not sensitive to pertussis toxin. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 175:25–30.
35. Rime, H., O. Haccard, and R. Ozon. 1992. Activation of $p34^{cdc2}$ kinase by cyclin is negatively regulated by cyclic amp-dependent protein kinase in *Xenopus* oocytes. *Dev. Biol.* 151:105–110.
36. Sadler, S. E., and J. L. Maller. 1983. Inhibition of *Xenopus* oocyte adenylate cyclase by progesterone and 2,5′-dideoxyadenosine is associated with slowing of guanine nucleotide exchange. *J. Biol. Chem.* 258:7935–7941.
37. Sadler, S. E., and J. L. Maller. 1989. A similar pool of cyclic AMP phosphodiesterase in *Xenopus* oocytes is stimulated by insulin, insulin-like growth factor I, and [Val^{12} Thr^{59}] Ha-ras protein. *J. Biol. Chem.* 264: 856–861.
38. Sadler, S., J. O. Maller, and J. B. Gibbs. 1990. Transforming *ras* proteins accelerate hormone-induced maturation and stimulate cyclic AMP phosphodiesterase in *Xenopus* oocytes. *Mol. Cell. Biol.* 10:1689–1696.
39. 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 (Lond.).* 335:519–525.
40. Sagata, N., I. Daar, M. Oskarsson, S. D. Showalter, and G. F. Vande Woude. 1989. The product of the *mos* proto-oncogene is a candidate "initiator" for oocyte maturation. *Science (Wash. DC).* 245:643–646.
41. 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 (Lond.).* 342:512–518.
42. Schorderet-Slatkine, S. M. Schorderet, and E. E. Baulieu. 1982. Cyclic AMP-mediated control of meiosis: effects of progesterone, cholera toxin, membrane-active drugs in *Xenopus* laevis oocytes. *Proc. Natl. Acad. Sci. USA.* 79:850–854.
43. Schultz, R. M., R. R. Montgomery, and J. R. Belanoff. 1983. Regulation of mouse oocyte meiotic maturation: implication of a decrease in oocyte cAMP and protein dephosphorylation in commitment to resume meiosis. *Dev. Biol.* 97:264–273.
44. Smith, L. D. 1989. The induction of oocyte maturation: transmembrane signalling events and regulation of the cell cycle. *Development.* 107:685–699.
45. Spivak, J. G., R. L. Erikson, and J. Maller. 1984. Microinjection of $pp60^{v\text{-}src}$ into *Xenopus* oocytes increases phosphorylation of ribosomal protein S6 and accelerates the rate of progesterone-induced meiotic maturation. *Mol. Cell. Biol.* 4:1631–1634.
46. Sturgill, T. W., L. B. Ray, E. Erikson, and J. L. Maller. 1988. Insulin-stimulated MAP-2 kinase phosphorylates and activates ribosomal protein S6 kinase II. *Nature (Lond.).* 334:715–718.
47. Tash, J. S., L. Lagace, D. R. Lynch, S. M. Cox, B. R. Brinkley, and A. R. Means. 1981. Role of cAMP-dependent protein phosphorylation in microtubule assembly and function. *In* Protein Phosphorylation, Book B, (Rosen, D. M., and E. G. Krebs, editors). Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
48. Thomas, G., J. Martin-Perez, M. Siegman, and A. Otte. 1982. The effect of serum, EGF, PGF_{2A} and insulin on S6 phosphorylation and the initiation of protein and DNA synthesis. *Cell.* 30:235–242.
49. Urner, R., W. L. Hermann, E. E. Balieu, and S. Schorderet-Slatkine. 1983. Inhibition of denuded mouse oocyte meiotic maturation by forskolin, an activator of adenylate cyclase. *Endocrinology.* 113:1170–1172.
50. Yew, N., M. L. Mellini, and G. F. Vande Woude. 1992. Meiotic initiation by the *mos* protein in *Xenopus*. *Nature (Lond.).* 355:649–652.
51. Zhou, R., M. Oskarsson, R. S. Paules, N. Schulz, D. Cleveland, and G. F. Vande Woude. 1991. Ability of the c-*mos* product to associate with and phosphorylate tubulin. *Science (Wash. DC).* 251:671–675.