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Cap ribose methylation of c-*mos* mRNA stimulates translation and oocyte maturation in *Xenopus laevis*

Hideaki Kuge+, George G. Brownlee[1], Paul D. Gershon[2] and Joel D. Richter*

Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, MA 01655, USA, [1]Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, UK and [2]Department of Biochemistry and Biophysics, Institute of Bioscience and Technology, Texas A & M University, Houston, TX 77030, USA

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ABSTRACT

In *Xenopus* oocytes, progesterone stimulates the cytoplasmic polyadenylation and resulting translational activation of c-*mos* mRNA, which is necessary for the induction of oocyte maturation. Although details of the biochemistry of polyadenylation are beginning to emerge, the mechanism by which 3′ poly(A) addition stimulates translation initiation is enigmatic. A previous report showed that polyadenylation induced cap-specific 2′-*O*-methylation, and suggested that this 5′ end modification was important for translational activation. Here, we demonstrate that injected c-*mos* RNA undergoes polyadenylation and cap ribose methylation. Inhibition of this methylation by S-isobutylthioadenosine (SIBA), a methyltransferase inhibitor, has little effect on progesterone-induced c-*mos* mRNA polyadenylation or general protein synthesis, but prevents the synthesis of Mos protein as well as oocyte maturation. Maturation can be rescued, however, by the injection of factors that act downstream of Mos, such as cyclin A and B mRNAs. Most importantly, we show that the translational efficiency of injected mRNAs containing cap-specific 2′-*O*-methylation (cap I) is significantly enhanced compared to RNAs that do not contain the methylated ribose (cap 0). These results suggest that cap ribose methylation of c-*mos* mRNA is important for translational recruitment and for the progression of oocytes through meiosis.

INTRODUCTION

The c-*mos* proto-oncogene product plays a key role in the control of vertebrate oocyte meiosis (1,2). In *Xenopus* oocytes, Mos appears to have at least three functions: (i) it stimulates re-entry into the meiotic divisions (oocyte maturation) (3,4); (ii) it suppresses DNA replication after meiosis I (5); and (iii) it promotes meiotic arrest after meiosis II (6). Mos is a serine/threonine kinase that initiates a cascade of events culminating in the activation of maturation promoting factor (MPF), a heterodimer composed of $p34^{cdc2}$ kinase and cyclin B. It is active MPF that is directly responsible for the morphological changes that occur during maturation, such as chromatin condensation and germinal vesicle breakdown (1,2). Although full-grown oocytes have no Mos protein, they do contain translationally dormant c-*mos* mRNA that is activated soon after the oocytes are exposed to progesterone, the primary stimulus of maturation (3). Translational control of c-*mos* RNA, therefore, is an essential regulatory step in early development.

c-*mos* is one of several mRNAs whose translation is induced by cytoplasmic polyadenylation (7,8). In this process, quiescent mRNAs in oocytes have relatively short poly(A) tails, usually fewer than 20 nt. Following the induction of maturation by progesterone, the poly(A) tails of these messages are elongated and translation ensues (9). Two *cis*-acting elements in the 3′ untranslated regions (UTRs) of responding mRNAs are necessary for cytoplasmic polyadenylation in maturing oocytes: the consensus sequence UUUUUAU (cytoplasmic polyadenylation element, CPE) and the polyadenylation hexanucleotide AAUAAA (9–11). The factor that binds the CPE, CPEB (12–14), is necessary for polyadenylation (13,15), and may act by recruiting additional factors to the AAUAAA (16), which in turn probably recruits the poly(A) polymerase.

Recent evidence demonstrates an interesting link between 3′ poly(A) addition and a 5′ cap-specific modification, and provides a clue as to how maternal mRNA translation could be regulated. Using oocyte histone B4 mRNA as a model, Kuge and Richter (17) showed that the cap 0 structure (^{7m}GpppN) of this transcript was converted, in a maturation- and polyadenylation-dependent manner, into cap I (^{7m}GpppN$_m$) and cap II (^{7m}GpppN$_m$N$_m$) forms by 2′-*O*-methylation of the penultimate and third nucleotides. The inhibition of these modifications substantially lowered the translational activation of this mRNA during oocyte maturation (17). Whether cap ribose methylation has important implications for early development, or whether it is sufficient to stimulate translation in the absence of polyadenylation, was not addressed.

In this study, we have explored these questions by examining the possible cap ribose methylation of c-*mos* mRNA. Injected c-*mos* RNA undergoes polyadenylation and cap ribose methylation. S-isobutylthioadenosine (SIBA), a methyltransferase inhibitor,

*To whom correspondence should be addressed. Tel: +1 508 842 8921; Fax: +1 508 842 3915; Email: joel.richter@banyan.ummed.edu

+Present address: Department of Biochemistry, Kochi Medical School, Kochi 783, Japan

has little effect on c-*mos* RNA polyadenylation or general protein synthesis, but completely abrogates 2′-*O*-methylation of this RNA, and prevents Mos synthesis and oocyte maturation. However, the injection of cyclin mRNAs, which act downstream of Mos, rescues oocyte maturation to normal levels, indicating the lack of a non-specific toxic effect by SIBA. We have also prepared cap 0- and cap I-containing luciferase and c-*mos* mRNAs *in vitro* and injected them into oocytes. A comparison of the translation of these mRNAs demonstrates that cap I is a potent enhancer of translation and oocyte maturation. The implications of cap-specific 2′-*O*-methylation for translation and early development are discussed.

MATERIALS AND METHODS

Oocytes

Xenopus oocytes were isolated as described (17) and cultured in Bath's medium in the absence or presence of 10 µM progesterone. For the inhibitor studies, oocytes were pre-incubated with the indicated concentrations of SIBA for 2 h. After injection of RNA, the oocytes were cultured for 6 h in the presence of the inhibitor.

RNA and protein

The RNA substrates for the methylation and polyadenylation assays were synthesized by SP6 RNA polymerase with the inclusion of cap analog (^{7m}GpppG) and [α-^{32}P]GTP (3000 Ci/mmol) (18). Plasmid DNA for substrate B was constructed by joining the *Bbs*I–*Eco*RI fragment of pXmos8 (3) to *Hin*dIII and *Eco*RI-digested pSP64 (Promega) through one blunt-end ligation. The second base from the transcription start site of this DNA was changed from adenine to guanine as described (17) for templates A and S. Before transcription, DNAs were digested with *Eco*RI (templates A and B) or *Ssp*I (for substrate S). Clam cyclin A and B mRNAs were synthesized as described (19). The polyadenylation and methylation assays have been presented (17). Briefly, for the methylation assay, total RNA was recovered from injected oocytes and divided into two portions (one for the methylation assay and the other for the polyadenylation assay), digested to completion with RNase T2 and then with calf intestinal alkaline phosphatase. The digests were separated on a 20% acrylamide/8 M urea gel and analyzed on a phosphorimager. The cap I marker for the methylation assay was generated by adding a cap structure to a 2′-*O*-ribose methylated RNA oligomer (ppG_mAAUACUCAAG) with guanylyltransferase (BRL) and [α-^{32}P]GTP (20). To quantify methylation efficiency, the radioactivity in the polyadenylated RNA with a tail of >20 residues was measured in a phosphorimager after gel electrophoresis, and was compared to the radioactivity in the cap I and cap II bands. Because 2.2% of the [α-^{32}P]GMP in the RNA is in the cap, the proportion yields the percentage of cap specific 2′-*O*-methylation. Furthermore, the quantification of polyadenylation and cap ribose methylation was derived from RNA extracted from the same group of injected oocytes, and thus there is no difference in sample recovery.

Western analysis with Mos antibody (Santa Cruz) was described (22). Protein synthesis in SIBA-treated oocytes was measured by trichloroacetic acid precipitation of protein following metabolic labeling with [^{35}S]methionine (1000 Ci/mmol) (17).

Preparation of cap I

The capped ^{7m}Gppp-oligoribonucleotides were prepared by adding cap to a chemically-synthesized diphosphorylated RNA-oligomer (ppGAAUACUCAAG) or to a 2′-*O*-ribose methylated RNA-oligomer (ppG_mAAUACUCAAG) (20) by guanylyltransferase (BRL) in 50 mM Tris–HCl pH 7.9, 1.25 mM $MgCl_2$, 6 mM KCl, 2.5 mM DTT, 4 U/µl RNasin (Promega), 0.5 pmol/µl RNA oligo, 6 mCi/ml [α-^{32}P]GTP, 5 µM S-adenosylmethionine (SAM) and 0.5 U/µl guanylyltransferase for 45 min at 37°C as described (20). Plasmid DNA for the luciferase coding region was created by ligating the *Sac*I–*Xba*I fragment of pGL3-basic (Promega) to *Sac*I and *Xba*I-digested pBluescript(KS) (Stratagene). Plasmid DNA for the c-*mos* coding region was created by ligating the *Eco*RI–*Sal*I fragment of pMALcRI-Xe (22) to *Eco*RI and *Sal*I digested pBluescript(SK). This plasmid DNA was linearized with *Eco*RI before transcription with T3 RNA polymerase. The 5′ ends of luciferase and c-*mos* RNAs were converted into monophosphate form by treatment with tobacco acid pyrophosphatase (TAP) (Epicentre) in 50 mM sodium acetate pH 5.0, 0.1% β-mercapto-ethanol, 1 mM EDTA, 0.01% Triton X-100, 0.1 pmol/µl RNA and 0.3 U/µl TAP for 2 h at 37°C. Ligation of a capped RNA oligomer to the luciferase or c-*mos* RNA was performed as described (23) with some modifications. The ligation conditions were: 33 mM Tris–acetate, pH 7.8, 66 mM potassium acetate, 10 mM magnesium acetate, 20 mM dithiothreitol, 1 mM ATP, 0.5 mM capped RNA oligomer, 0.5 µM luciferase or c-*mos* RNA, 0.5 µM bridge DNA oligomer (CCAATTCGCCCCTTGAGTATTC for luc) or (CTTTTGTTCCCCTTGAGTATTC for c-*mos*), 1 U/µl T4 DNA ligase (Epicentre), incubated for 12 h at 16°C. The DNA was then removed by DNase treatment.

Generation of ^{7m}G antisera and immunoselection of RNA

Antigen preparation to generate rabbit immune serum against ^{7m}G has been presented (24). Briefly, ^{7m}G was conjugated to BSA by dissolving the nucleotide in 0.1 M $NaIO_4$, and then removing excess $NaIO_4$ with ethylene glycol. This was added to BSA and mixed for 45 min using 5% potassium carbonate to maintain pH 9. Following a further incubation in $NaBH_4$, formic acid was added and the pH adjusted to 8.5 with NH_4OH. The mixture was dialyzed and used to immunize rabbits. Capped RNA was immunoselected with antibody bound to protein A Sepharose essentially as described by Yang *et al*. (25). The cap structure of immunoselected RNA was analyzed by digestion with nuclease P1 and cellulose TLC (26).

Measurement of translational efficiency

The stability and translation of the ligated luciferase mRNA in oocytes was measured by injecting 10 pg mRNA into oocytes in the absence of progesterone. After a 6 h incubation, RNA was extracted and analyzed by electrophoresis on a 5% polyacrylamide/8 M urea gel and phosphorimaging. Luciferase assays were performed with a kit (Promega) following the manufacturer's instructions.

RESULTS

Progesterone induces c-*mos* mRNA cap ribose methylation

A portion of the c-*mos* 3′ UTR containing both the CPE and hexanucleotide AAUAAA was synthesized *in vitro* in the presence of both [α-^{32}P]GTP and ^{7m}GpppG to provide a capped,

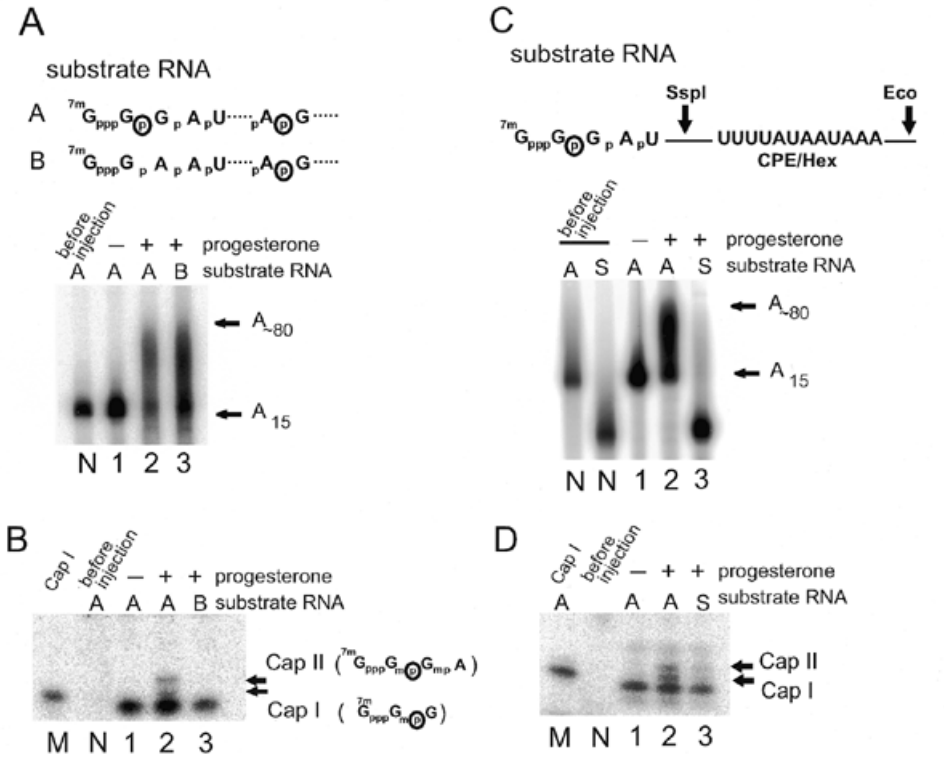

Figure 1. Progesterone induces polyadenylation and cap ribose methylation of c-*mos* mRNA. Polyadenylation (**A**) and cap ribose methylation (**B**) of c-*mos* mRNA were examined by injection of substrate RNA A (lanes 1 and 2) or B (lane 3), which contain a part of 3′UTR of c-*mos* mRNA, into oocytes in the absence (lane 1) or presence (lanes 2 and 3) of progesterone. The circled p refers to the radioactive phosphate labeled by [α-^{32}P]GTP. Polyadenylation (**C**) and cap ribose methylation (**D**) were also examined with a wild type RNA (DNA template cleaved with *Eco*RI, substrate A) (lanes 1 and 2) or a part of the 3′UTR of c-*mos* mRNA that lacks CPE/ Hex (DNA cleaved with *Ssp*I, substrate S) (lane 3). Lane N shows the assays performed on substrates A without injection. Lane M shows the molecular marker of cap I. In (B) and (D), a band that migrates faster than cap I is evident. Although the origin of this band is unknown, it is not derived from the phosphate between the second and third bases and is not progesterone-dependent.

internally-labeled transcript (Fig. 1A, substrate A). Another, nearly identical, RNA was synthesized in the same manner, except that it contained an adenine for guanine substitution at the third nucleotide (counting the terminal 7-methylguanosine as the first) (substrate B). Following injection into oocytes, both RNAs underwent progesterone-induced polyadenylation, receiving a maximum of ~80 adenosine residues (Fig. 1A, lanes 2 and 3).

To examine cap ribose methylation, we used an assay based on the resistance 2′-*O*-methylation confers to RNase T2 hydrolysis (17). Total RNA from oocytes injected with the ^{32}P-labeled RNAs was digested to completion with RNase T2, followed by treatment with alkaline phosphatase to remove 3′ phosphates. The products were resolved on a denaturing 20% polyacrylamide gel and visualized on a phosphorimager. Figure 1B shows that non-injected substrate A RNA was completely hydrolyzed by the nuclease (lane N), but yielded a band of unknown origin when injected into oocytes (lane 1). However, following progesterone-induced oocyte maturation, two new bands, corresponding in mobility to cap I and cap II, were detected (lane 2). To confirm that these bands were derived from the cap structure, injected substrate B RNA was subjected to the same treatment. The two bands were not detected in oocytes treated with progesterone (lane 3). Because this RNA contained the same radiolabeled phosphates as substrate A, with the exception of the one between the second and third nucleotides, we conclude that the two new bands represent cap I and cap II. By comparing the radioactivity in the polyadenylated RNA with the radioactivity in the bands of cap I and cap II, we estimate that 45% of the injected cap 0-containing form of RNA became 2′-*O*-methylated.

Template DNAs were also linearized with *Eco*RI (substrate A) or *Ssp*I (substrate S) so that the resulting transcripts do or do not contain the CPE and hexanucleotide (Fig. 1C). As expected, RNA from the *Eco*RI-digested template underwent progesterone-induced polyadenylation (Fig. 1C, lane 2) and cap ribose methylation (Fig. 1D, lane 2). However, elimination of the CPE and hexanucleotide abolished polyadenylation (Fig. 1C, lane 3) and significantly decreased cap ribose methylation (Fig. 1D, lane 3). These results show that the 3′ UTR of injected c-*mos* RNA controls both polyadenylation and cap ribose methylation. A previous study with B4 RNA showed that a block of the 3′ end by cordycepin inhibited both polyadenylation and cap ribose methylation (17), suggesting that the latter modification is dependent upon the former.

Inhibition of cap ribose methylation by SIBA blocks translational activation of endogenous c-*mos* mRNA

To investigate the possible involvement of cap ribose methylation in the translation of c-*mos* mRNA, oocytes were incubated with SIBA, a methyltransferase inhibitor (17). Figure 2A shows that progesterone induced the polyadenylation of c-*mos* RNA at all

concentrations of SIBA tested, although there was some decrease in the length of the steady-state tail at the higher concentrations of SIBA. SIBA slightly inhibited cap ribose methylation at 0.15 mM, but completely abolished it at 0.5 and 0.75 mM (Fig. 2B). A western blot probed with Mos antibody shows that although this protein was not detected in non-maturing oocytes, progesterone induced its steady-state level to a readily observable amount (Fig. 2C, lanes 1 and 2). Although up to 0.15 mM SIBA had little effect on Mos levels (lanes 3–5), it completely prevented Mos accumulation when present at 0.5 and 0.75 mM (lanes 6 and 7). SIBA had no significant effect on general protein synthesis irrespective of the concentration used (Fig. 2D). Consistent with Mos synthesis being necessary for progesterone-induced oocyte maturation (3), germinal vesicle breakdown (GVBD), an indicator of oocyte maturation, was inhibited by SIBA (Fig. 2E). These results indicate that polyadenylation can be uncoupled from translation by inhibiting cap ribose methylation, and suggests a causal link between this 5′ end modification and c-*mos* mRNA translation.

Cyclin A and B mRNAs rescue the SIBA block to oocyte maturation

To determine whether the above results could be due to toxic effects of SIBA, rescue experiments were attempted by injecting molecules that act downstream of Mos (Fig. 3). Although oocytes incubated with SIBA and progesterone did not undergo GVBD (lane 2), the injection of *in vitro* synthesized clam cyclin A and B mRNAs (both having cap 0) into oocytes in the presence of SIBA nearly completely restored the high incidence of GVBD (lanes 3 and 4). Cyclin B mRNA contained neither a CPE nor an AAUAAA hexanucleotide, and hence was not polyadenylated or cap ribose methylated (data not shown). Thus, the translational machinery, as well as the downstream biochemical cascade from MPF activation to GVBD, remained functional in SIBA-treated oocytes.

Cap ribose methylation stimulates translation

To address the importance of cap ribose methylation in translational regulation without the use of inhibitors, we compared the translational efficiencies of cap 0- and cap I-terminated versions of mRNA. To prepare these, we developed a regimen that includes the chemical synthesis of an RNA oligomer containing a 5′-terminal 2′-*O*-methylated nucleotide (for cap I) (20), followed by enzymatic capping (20), and ligation to the luciferase reporter mRNA (23) (Fig. 4A). In step 1, an RNA oligomer was prepared containing a 2′-*O*-methylated 5′ nucleotide and a 5′ diphosphate. Due to impurities, 5′ monophosphorylated and 5′ hydroxylated oligomers were also present. Although only the 5′ diphosphate can serve as a substrate, the entire oligomer mixture was incubated with guanylyltransferase, [α-^{32}P]GTP and S-adenosylmethionine (SAM). For step 2, luciferase mRNA was synthesized enzymatically *in vitro*, and treated with pyrophosphatase to convert 5′ terminal triphosphates into monophosphate form. In step 3, the RNA oligomer and luciferase mRNA were ligated with T4 DNA ligase using a bridging DNA oligomer to correctly position the two RNA molecules. In step 4, the desired chimeric RNA containing cap I or cap 0 was purified with ^{7m}G-specific antibody.

To ensure that this antibody was ^{7m}G-specific, RNA oligomers were capped with [α-^{32}P]GTP and SAM. They were immunoprecipitated with the ^{7m}G antibody, digested with nuclease P1, and the products resolved by TLC and visualized by phosphorimaging

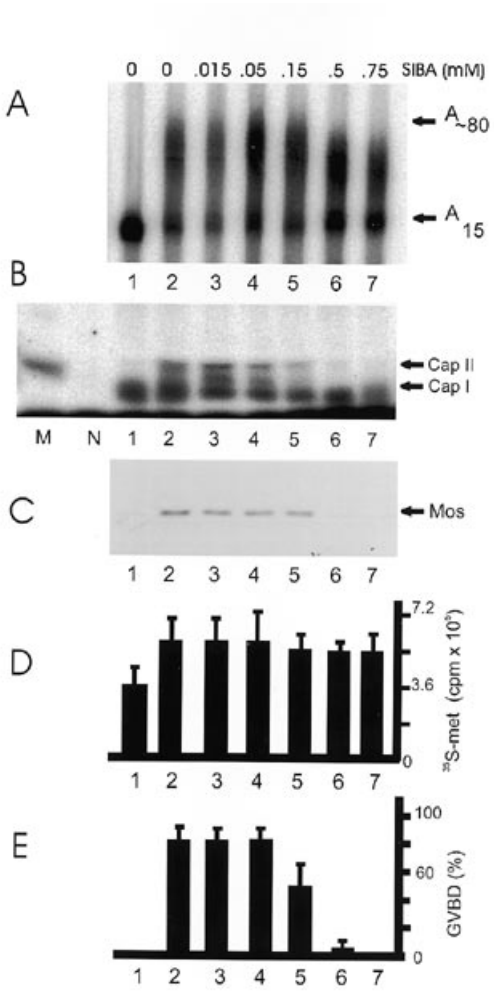

Figure 2. Inhibition of cap ribose methylation by SIBA abolishes progesterone-induced *Mos* synthesis and oocyte maturation. Oocytes were treated with the indicated concentrations of SIBA in the absence (lane 1) or presence (lanes 2–6) of progesterone, and analyzed for the polyadenylation (**A**) and methylation (**B**) of injected c-*mos* RNA. Lanes M and N are as indicated in Figure 1B. In parallel, the synthesis of endogenous Mos (**C**), or protein in general (**D**), were examined by a western blot probed with Mos antibody, and metabolic labeling of oocytes with [^{35}S]methionine, respectively. GVBD after 6 h of progesterone treatment was scored by the appearance of a white spot at the animal pole (**E**). The bars represents the mean ± SD (three experiments).

(Fig. 4B). Before immunoprecipitation, both ^{7m}GpppG and GpppG were detected because of incomplete base methylation (lane 1), but only the former was precipitated with the antibody (lane 2). Thus, immunoprecipitation with anti-^{7m}G antibody isolates the authentic cap structure. The antibody also captures large capped mRNAs because only radiolabeled luciferase mRNAs ligated with cap 0- or cap I-containing oligomers, but not with an oligomer without a cap, were immunoprecipitated in similar experiments (Fig. 4C, lanes 2 and 3).

Identical amounts of radiolabeled poly(A)-deficient chimeric luciferase mRNAs containing cap 0 or cap I were injected into oocytes. RNA was extracted 6 h later and the relative stability of the two luciferase messages was shown to be equivalent (Fig. 5). Translation, as measured by luciferase activity, was on average 4.4-fold greater in oocytes injected with mRNA containing cap I

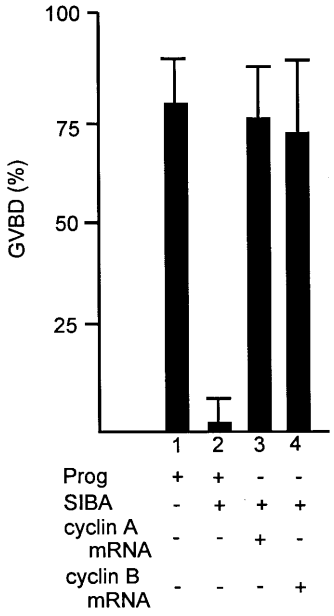

Figure 3. Cyclin A and B mRNAs induce GVBD in the presence of SIBA. Oocytes were pre-incubated with control medium (lane 1) or medium containing 0.5 mM SIBA (lanes 2–4), followed by incubation with progesterone (10 μM, lane 1), or injection of 5 ng cyclin A mRNA (lane 3) or 5 ng cyclin B mRNA (lane 4). GVBD was scored as in Figure 2.

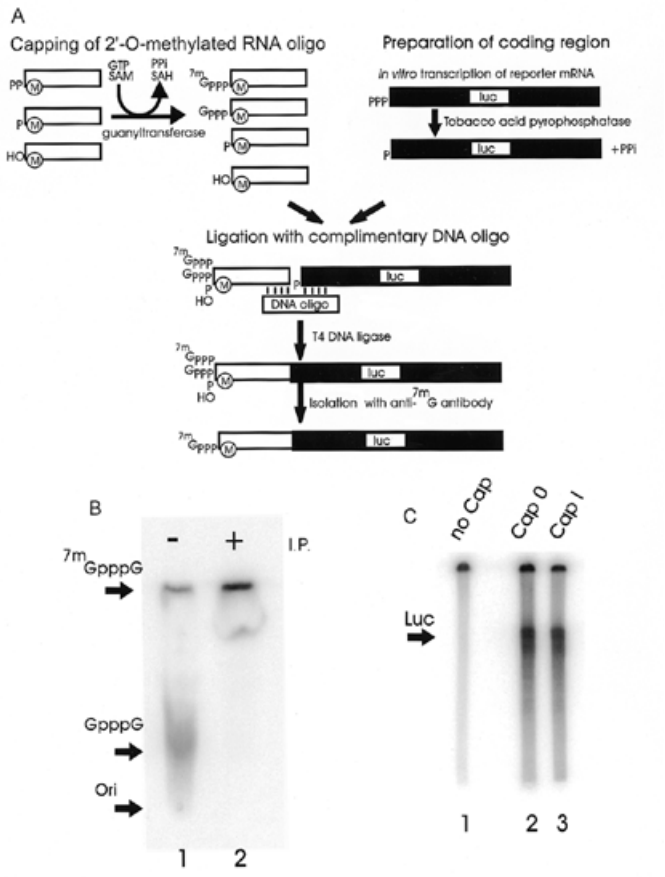

Figure 4. Preparation of mRNA containing cap I. (**A**) Schematic diagram of the preparation of a reporter mRNA with 2′-*O*-methylated cap. The capped ^{7m}Gppp-oligoribonucleotides were prepared by adding a cap structure to a chemically synthesized, diphosphorylated and 2′-*O*-ribose methylated RNA-oligomer by guanylyltransferase with GTP and SAM. The circled M refers to 2′-*O*-ribose methylation, and p and HO refer to contaminating oligonucleotides with monophosphate and hydroxyl 5′ ends. The 5′ end of luciferase RNA was converted into monophosphate form by treatment with tobacco acid pyrophosphatase. Ligation of a capped RNA oligomer to the luciferase RNA was performed with T4 DNA ligase in the presence of complementary bridging DNA oligomer. After the ligation, the desired products were immunoselected with anti-^{7m}G antibody. (**B**) Anti-^{7m}G antibody isolates ^{7m}GpppG from GpppG. 5′ diphosphorylated RNA oligomer was capped by guanylyltransferase with SAM and [α-^{32}P]GTP. The product was digested with nuclease P1 before (lane 1) or after (lane 2) isolation with ^{7m}G antibody, and analyzed by cellulose TLC. (**C**) ^{7m}G antibody selects the ligation product from non-ligated coding region. Radiolabeled luciferase coding region was ligated without capped RNA oligomer (lane 1) or with cap 0 RNA oligomer (lane 2) or cap I RNA oligomer (lane 3), immunoselected with ^{7m}G antibody, and analyzed on a polyacrylamide gel.

compared to cap 0. These data demonstrate that cap ribose methylation increases translation.

Similar injection experiments were performed with c-*mos* mRNA ligated to the capped oligomers, but here the incidence of oocyte maturation, in the absence of progesterone, was determined (Fig. 6A). When 0.1 ng of these poly(A)-deficient messages was injected, only the c-*mos* mRNA containing cap I induced maturation (10% of oocytes). The injection of 0.3 or 1 ng/oocyte greatly increased the incidence of maturation, but at these doses, the cap 0-containing RNA also induced maturation, although at less than one-half the efficiency of cap I-containing RNA. In addition, the relative stability of injected c-*mos* mRNA containing cap 0 or cap I was the same (Fig. 6B, bottom). Therefore, cap ribose methylation not only can enhance translation, but oocyte maturation as well.

DISCUSSION

Four main conclusions can be drawn from this study: (i) injected c-*mos* mRNA 3′ UTR undergoes cap ribose methylation during oocyte maturation; (ii) polyadenylation and translation of c-*mos* mRNA can be uncoupled by a methyltransferase inhibitor; (iii) cap ribose methylation enhances translation *in vivo* in the absence of poly(A); and (iv) cap ribose methylation enhances the rate of oocyte maturation by c-*mos* mRNA in the absence of progesterone.

One key reagent used in these studies was SIBA, a stable analogue of the SAM metabolite S-adenosylhomocysteine that is an inhibitor of methyltransferase reactions (27). While SIBA had little effect on c-*mos* mRNA polyadenylation or overall protein synthesis, it abolished the cap ribose methylation of this message and prevented both Mos synthesis and oocyte maturation in a similar dose-dependent manner. However, because it was formally possible that SIBA had some non-specific effect that obviated oocyte maturation, we performed rescue experiments by injecting two *in vitro* synthesized cyclin mRNAs, which act downstream of Mos. Both mRNAs consistently induced oocyte maturation, demonstrating that SIBA had no deleterious influence on the ability of oocytes to translate mRNA, or any of the metabolic reactions that lead to oocyte maturation. These data suggest that c-*mos* mRNA translation, and resulting oocyte maturation, is dependent upon cap ribose methylation. Finally, and most importantly, biochemical and biological assays used to test the *in*

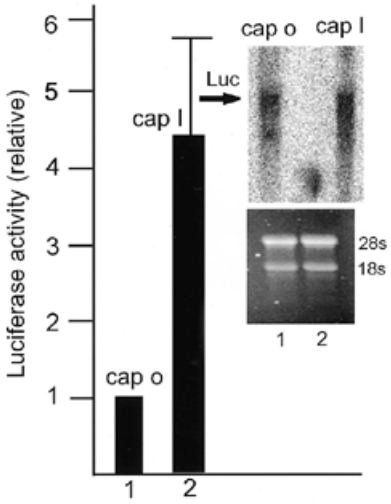

Figure 5. Cap ribose methylation enhances translation in the absence of poly(A). Radiolabeled luciferase mRNAs with cap 0 (lane 1) or cap I (lane 2) were injected into oocytes without progesterone treatment. Total RNA was isolated 6 h later, and the labeled RNA was analyzed by gel electrophoresis and phosphorimaging. In addition, the overall quality of extracted RNA is shown by ethidium bromide staining (inset). Translational efficiency of the luciferase mRNAs with cap 0 (lane 1) or cap I (lane I) were measured in oocytes without progesterone treatment. The mean of six experiments ± SD is presented. The 4.4-fold difference is statistically significant ($P < 0.01$).

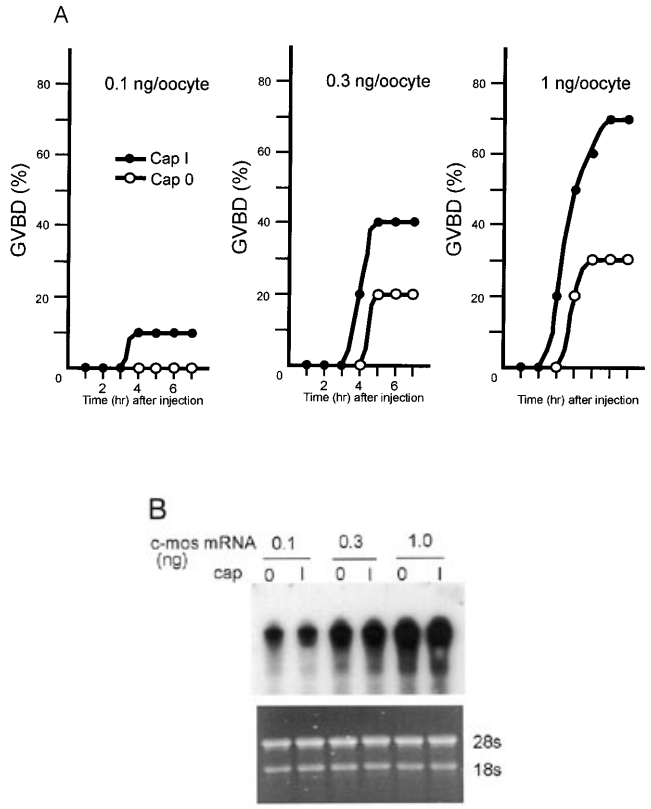

Figure 6. c-*mos* mRNA with cap I is an effective inducer of oocyte maturation. (**A**) Three concentrations of c-*mos* mRNA containing cap 0 or cap I, but no poly(A) tail, were injected into oocytes with no progesterone treatment. GVBD was scored by the appearance of a white spot at the animal pole. (**B**) Other oocytes were injected with radiolabeled cap 0 or cap I-containing c-*mos* mRNA, which was then extracted and analyzed. In addition, the overall quality of the extracted RNA was determined by ethidium bromide staining of rRNA, which also served as a loading control.

vivo translational efficiencies of two mRNAs containing cap 0 and cap I showed that cap-specific 2′-*O*-methylation enhances translation in the absence of poly(A).

Cap I is an enhancer of translation

Luciferase mRNA with cap I is translated 4.4-fold more efficiently than with cap 0. This stimulation is about the same as that which occurs with injected mRNAs that are poly(A) elongated during oocyte maturation (9,28). In contrast, poly(A) (29) and 2′-*O*-methylation (30) enhance translation by <50% in reticulocyte lysates, and point to the fact that *in vitro* translation systems can be notorious for their lack of regulation. In addition, this could be due to an excess translational capacity *in vitro*, whereas in oocytes, message levels far exceed the cell's protein synthesis capabilities (31). Finally, we should note that both cap I and cap II structures are detected in mature oocytes (e.g. Fig. 1), and one might surmise that luciferase mRNA with cap II would be translated more efficiently than with cap I. Unfortunately, our attempts to construct such an mRNA were not successful.

Because some studies have shown that a poly(A) can enhance translation oocytes (e.g. 32), we injected a luciferase mRNA that contained both cap I and a poly(A) tail. However, the translational enhancement was not additive with the two modifications (data not shown). This may be related to the time the oocytes are cultured (32), or to the possibility that not all reporter mRNAs associate with the translational apparatus in an identical manner.

Although cap ribose methylation enhances the translation of injected c-*mos* mRNA, it is obviously not essential (Fig. 6) (this message would not significantly undergo cap ribose methylation after injection because it lacks a CPE and hexanucleotide). This is probably due to the fact that almost any mRNA will be translated to some extent following injection, and likely reflects the alacrity with which ribosomes associate with newly introduced message. In addition, oocyte maturation is an all-or-none event, so that if even if a small amount of Mos is synthesized, oocyte maturation will be triggered. Hence, the data from the luciferase assay (Fig. 5) is a more quantitative indictor of relative translational control.

Injected c-*mos* mRNA, like injected Mos protein (3,4), is an efficient inducer of oocyte maturation. Moreover, it has been shown that Mos protein induces maturation in oocytes incubated in the presence of cycloheximide (reviewed in 2), which indicates that this protein is all that is necessary for maturation. However, oocytes incubated in SIBA and injected with Mos do not mature (H.Kuge and J.D.Richter, unpublished data), even though they are competent to do so (Fig. 3). Thus, there could be another factor involved in maturation, which we suggest could be regulated at the level of polyadenylation-induced cap ribose methylation.

How cap ribose methylation stimulates translation is unclear, but one factor that might be involved is eIF-4E, the cap binding protein. The three-dimensional structure of cocrystals of eIF-4E and ^{7m}GDP has been examined (33). Like vaccinia VP39, a viral

cap-specific methyltransferase (34), the protein contains a cleft that could accommodate ^{7m}GpppN, where N is any nucleotide, and thus could potentially recognize a methylated ribose moiety of cap I and cap II. It is also possible that different isoforms of eIF-4E recognize cap I, which could stimulate translation of specific messages. For example, the phosphorylation of eIF-4E increases its affinity for the cap (35), which may be potentiated by ribose methylation. Experiments are underway to determine the role eIF-4E might play in cap I-enhanced translation.

Another question is how 3′ poly(A) addition stimulates cap ribose methylation. Prior studies show that the mRNA cap-specific 2′-*O*-methyltransferase of vaccinia virus is encoded by the same polypeptide chain as the vaccinia poly(A) polymerase processivity factor (26,36), and that it heterodimerizes specifically with the viral poly(A) polymerase catalytic subunit (37). This highlights the possibility of an advantageous connection between poly(A) tail elongation and cap-specific 2′-*O*-methylation in the cytoplasm of eukaryotic cells, perhaps even oocytes. This is, if the oocyte poly(A) polymerase is also bound to a cap-specific ribose methyltransferase, one could imagine message circularity as the two ends of the mRNA come into close proximity mediated by the enzymes. Therefore, only mRNAs undergoing polyadenylation will be cap ribose methylated. This is consistent with the observation that only an mRNA in the active process of poly(A) addition undergoes cap ribose methylation (17). Clearly, the isolation and cloning of an oocyte cap-specific 2′-*O*-methyltransferase would help in determining whether this is the case.

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