Molecular Biology of the Cell
Vol. 8, 1633–1648, August 1997

A Dependent Pathway of Cytoplasmic Polyadenylation Reactions Linked to Cell Cycle Control by *c-mos* and CDK1 Activation

Scott Ballantyne, Donald L. Daniel, Jr., and Marvin Wickens*

Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706

Submitted February 11, 1997; Accepted April 21, 1997
Monitoring Editor: Tim Hunt

During oocyte maturation and early development, mRNAs receive poly(A) in the cytoplasm at distinct times relative to one another and to the cell cycle. These cytoplasmic polyadenylation reactions do not occur during oogenesis, but begin during oocyte maturation and continue throughout early development. In this report, we focus on the link between cytoplasmic polyadenylation and control of the cell cycle during meiotic maturation. Activation of maturation promoting factor, a complex of CDK1 and cyclin, is required for maturation and dependent on c-*mos* protein kinase. We demonstrate here that two classes of polyadenylation exist during oocyte maturation, defined by their dependence on c-*mos* and CDK1 protein kinases. Polyadenylation of the first class of mRNAs (class I) is independent of c-*mos* and CDK1 kinase activities, whereas polyadenylation of the second class (class II) requires both of these activities. Class I polyadenylation, through its effects on c-*mos* mRNA, is required for class II polyadenylation. *cis*-acting elements responsible for this distinction reside in the 3′-untranslated region, upstream of the polyadenylation signal AAUAAA. Cytoplasmic polyadenylation elements (CPEs) are sufficient to specify class I polyadenylation, and subtle changes in the CPE can substantially, though not entirely, shift an RNA from class I to class II. Activation of class I polyadenylation events is independent of hyperphosphorylation of CPE-binding protein or poly(A) polymerase, and requires cellular protein synthesis. The two classes of polyadenylation and of mRNA define a dependent pathway, in which polyadenylation of certain mRNAs requires the prior polyadenylation of another. We propose that this provides one method of regulating the temporal order of polyadenylation events, and links polyadenylation to the control of the meiotic cell cycle.

INTRODUCTION

Control of mRNA localization, translation, and stability play a prominent role in the regulation of key decisions during early development (Curtis *et al.*, 1995; Macdonald and Smibert, 1996; Wickens *et al.*, 1996). In *Drosophila* and *Caenorhabditis elegans*, for example, spatially restricted translational activation plays a critical role in the formation of anterior–posterior axes. Translational regulation is also interwoven with control of the cell cycle during the early development of many species. For example, in *Xenopus*, translational activation of c-*mos* mRNA is required for oocytes to progress through the meiotic cell cycle (Sagata *et al.*, 1988, 1989; Sheets *et al.*, 1995), whereas the ongoing translation of preexisting cyclin mRNAs is essential to propel mitoses after fertilization (Murray and Kirschner, 1989; Weeks *et al.*, 1991). Although the link between cell cycle control and the regulation of specific mRNAs is critical during early development, the underlying molecular mechanisms of the coupling have not been elucidated.

In the embryo, as in somatic cells, complexes between cyclins and cell division kinases (CDKs) drive the cell cycle (Murray and Hunt, 1993; Nigg, 1995). Meiotic maturation of *Xenopus* oocytes, in which cells advance from first to second meiosis, requires the

* Corresponding author.

activation of maturation (or M-phase) promoting factor (MPF), a complex of CDK1 and cyclin B. Translational activation of c-*mos* mRNA, which encodes a germ-cell-specific serine-threonine kinase, is needed for meiotic maturation (Sagata *et al.*, 1988), presumably because elevated levels of c-*mos* protein are necessary for activation of MPF (Sagata *et al.*, 1988, 1989; Yew *et al.*, 1992). During maturation and early cleavage, translation of several cyclin mRNAs (Kobayashi *et al.*, 1991) and CDK2 mRNA (Stebbins-Boaz and Richter, 1994) are stimulated. The elimination of specific cyclin B mRNAs disrupts mitoses after fertilization (Weeks *et al.*, 1991).

Regulation of poly(A) length provides a link between cell cycle control and translational regulation. Elongation of poly(A) tails on specific cytoplasmic mRNAs is often correlated with, and in some cases required for, their translational activation (reviewed in Richter, 1996; Wickens *et al.*, 1997). Removal of poly(A) typically is correlated with translational inactivation (Sturgess *et al.*, 1980; Dworkin *et al.*, 1985; Hyman and Wormington, 1988; Wormington, 1989; Paris and Philippe, 1990; Varnum and Wormington, 1990; Huarte *et al.*, 1992), and may be required for the turnover of the mRNA later in development (Audic *et al.*, in press). Cytoplasmic polyadenylation of c-*mos* mRNA is directly coupled to cell cycle control, in that it is required for meiotic maturation (Gebauer *et al.*, 1994; Sheets *et al.*, 1995).

Cytoplasmic polyadenylation requires *cis*-acting sequences in the 3′ untranslated region (3′-UTR) and a multicomponent polyadenylation apparatus (reviewed in Richter, 1996; Wickens *et al.*, 1997). Two sequences are critical: AAUAAA, the highly conserved signal for mRNA cleavage and polyadenylation in the nucleus; and a second sequence, termed a cytoplasmic polyadenylation element (CPE; Fox *et al.*, 1989; McGrew *et al.*, 1989; Salles *et al.*, 1992). The apparatus comprises at least three *trans*-acting factors: CPE-binding protein (CPEB), cleavage and polyadenylation specificity factor (CPSF), and poly(A) polymerase (PAP). CPEB, a 62-kDa protein found in the oocyte cytoplasm, is required for CPE-dependent polyadenylation in vitro and binds to CPE-containing RNAs (Hake and Richter, 1994; Stebbins-Boaz *et al.*, 1996). CPSF, a multi-subunit complex that interacts directly with AAUAAA, binds preferentially to CPE-containing RNAs, and, together with mammalian PAP, recapitulates CPE-dependent polyadenylation in vitro (Bilger *et al.*, 1994). Homologues of CPSF subunits are present in the oocyte cytoplasm (A. Bilger and M. Wickens, unpublished data). Both these cytoplasmic CPSF homologues and CPEB likely are parts of a cytoplasmic polyadenylation apparatus that acts on many mRNAs because they are required for polyadenylation of multiple mRNA substrates in vitro (Bilger *et al.*, 1994; Hake and Richter, 1994; Stebbins-Boaz *et al.*, 1996). Finally, PAP, the enzyme that polymerizes ATP into poly(A), has been identified in the *Xenopus* oocyte cytoplasm and is closely related in sequence to mammalian nuclear PAPs (Ballantyne *et al.*, 1995; Gebauer and Richter, 1995). Oocytes contain PAP mRNAs that correspond to full-length and C-terminally truncated forms of the mammalian nuclear enzyme (Ballantyne *et al.*, 1995; Gebauer and Richter, 1995).

Activation of cytoplasmic polyadenylation is coupled to resumption of the cell cycle. Cytoplasmic polyadenylation does not occur during oogenesis, but is activated only as the cell cycle resumes. In *Xenopus*, mice, and certain other species, it first occurs early in oocyte maturation; in other organisms, it begins with the onset of mitoses soon after fertilization. Once activated, cytoplasmic polyadenylation is highly regulated, such that specific mRNAs receive poly(A) at different times throughout early development. Although activation likely involves an increase in a CPE-dependent RNA-binding activity (Fox *et al.*, 1992), neither CPEB, PAP, nor $CPSF^{100}$ change dramatically in abundance as polyadenylation activity first appears (Hake and Richter, 1994; Ballantyne *et al.*, 1995; Bilger and Wickens, unpublished data). However, PAP and CPEB are phosphorylated during maturation, and either event might activate cytoplasmic polyadenylation (Hake and Richter, 1994; Ballantyne *et al.*, 1995).

MPF may link activation of polyadenylation to cell cycle control during meiotic maturation. Paris *et al.* (1991) proposed that MPF activates cytoplasmic polyadenylation by phosphorylating CPEB. In support of this view, cytoplasmic polyadenylation and CPEB phosphorylation can be induced in vivo and in vitro by overexpression of cyclin B or injection of MPF (McGrew and Richter, 1990; Paris *et al.*, 1991). However, activation of MPF requires prior cytoplasmic polyadenylation of c-*mos* mRNA (Sheets *et al.*, 1995; Barkoff *et al.*, unpublished data). In addition, hyperphosphorylation of mammalian PAP by MPF inactivates, rather than stimulates, its polyadenylation activity (Colgan *et al.*, 1996). These experiments suggest that activation of MPF requires cytoplasmic polyadenylation, not vice versa.

In this report, we examine the relationship in vivo between activation of cytoplasmic polyadenylation, c-*mos*, and CDK1 protein, the kinase component of MPF. We demonstrate that two classes of polyadenylation exist during oocyte maturation, defining two classes of mRNA. Polyadenylation of the first class of mRNA is independent of c-*mos* and CDK1 kinase, whereas polyadenylation of the second class requires both of these activities. CPEs within the mRNA can specify class I behavior, and can dramatically influence which pathway of activation is followed. The first class of cytoplasmic polyadenylation reactions is independent of detectable phosphorylation of CPEB and

Table 1. DNA oligonucleotides used in this study

Oligo	Sequence
1	5′-CGGATCCAAATCTGGTTTTTAATGTTTAATTCTATAAATAAAGTAAGTAATTGTTTTTTT
2	5′-TAGAAGAAAAAAAAAACAATTACTTACTTTATTTATAGAATTAAACATTAAAAACCAGA
3	5′-CGGATCCAAATCTGGTTTTTAATGTTTAATTC
4	5′-TTAAACATTAAAAACCAGATTTGGATCCGAGCT
5	5′-TATAAATAAAGCTCATTTTAACATGT
6	5′-CTAGACATGTTAAAATGAGCTTTATTTATAGAA
7	5′-CGGATCCTGTAAATAGTGTATTGTGTTTTTAATGTTTTACTGG
8	5′-TAAAACATTAAAAACACAATACACTATTTACAGGATCCGAGCT
9	5′-TTTTAATAAAGTAAGTAATTGTTTTTTTTTTCTT
10	5′-CTAGAAGAAAAAAAAAAACAATTACTTACTTTATTAAAACCAG
11	5′-CCGGGGGATCCACTAGTCTCGAGAAT
12	5′-ATTCTCGAGACTAGTGGATCCCCCGGGTAC
13	5′-ATTGCATGCCTGCAGAAGCTTGTTTTATAATAAAGAAATTGATTTGT
14	5′-CTAGACAAATCAATTTCTTTATTATAAAACAAGCTTCTGCAGGCATGCAAT
15	5′-GAAGCTTGTTTTTAATAAAGAAATTGATTTGTCTTCTAGAA
16	5′-AGCTTTCTAGAAGACAAATCAATTTCTTTATTAAAAACAAGCTTCTGCA
17	5′-CCCGAATTCATATGGCCTCCCACTGAAAG
18	5′-ATTTGTCGACCAATGTTGTTCCAATGTC
19	5′-AGCTTATTGAACTGCTTCATTTTC-3′
20	5′-CTGGGAAAATGAAGCAGTTCAATA-3′
21	5′-CCAGGTTCTTAACTTGTGATGGTGTTAAGTGTTTTTAATAAACTGACTTTACTCTCTAGAG-3′
22	5′-AATTCTCTAGAGAGTAAAGTCAGTTTATTAAAAACACTTAACACCATCACAAGTTAAGAAC-3′

PAP. The two classes of polyadenylation define a dependent pathway, in which polyadenylation of certain mRNAs requires prior polyadenylation of another. We propose that this provides a mechanism by which the temporal order of polyadenylation events are regulated and coupled to the progression of the meiotic cell cycle.

MATERIALS AND METHODS

Preparation of Radiolabeled Polyadenylation Substrates

Transcription in vitro. Capped, radiolabeled RNA for polyadenylation assays was prepared by in vitro transcription of appropriately digested plasmids using either SP6 or T7 RNA polymerase (Promega, Madison, WI) as indicated. Transcription reactions were performed using 1 μg of template in a 10-μl reaction as described (Fox *et al.*, 1989). RNA was purified and redissolved at a final concentration of 100 fmol/μl as described (Fox *et al.*, 1989).

Structure of RNA Substrates. The poly(A) site is designated as +1. Thus −83/+2 c-*mos* RNA contains 83 nts upstream of its poly(A) site and ends one nucleotide after its poly(A) site.

−83/+2 c-*mos* RNA (113 nts; Sheets *et al.*, 1994), 83/+2 cyclin B1 RNA (94 nts; Sheets *et al.*, 1994), −58/+1 H4 RNA (95 nts; Fox and Wickens, 1990), −242/+1 D7 RNA (277 nts; Fox *et al.*, 1989), and −141/−1 SV40 RNA containing UUUUUAU RNA (172 nts; Fox *et al.*, 1989) were synthesized as described in the citations provided.

−72/+1 cyclin A1 RNA. An insert containing the 3′ end of cyclin A1 (Kobayashi *et al.*, 1991) was created by annealing the DNA oligonucleotides 19 plus 20, and 21 plus 22 (see Table 1). The insert was cloned into pGEM4Z (Promega) that was cut with *Hin*dIII and *Eco*RI to create pGEM cyclin A1 −72/+1. The plasmid was cut with *Xba*I and transcribed with T7 RNA polymerase. The 86-nucleotide RNA contains 13 nucleotides of polylinker sequence, followed by 72 nucleotides of cyclin A1 sequence.

−59/−1 B4 RNA. An insert containing the 3′ end of B4 cDNA (Smith *et al.*, 1988) was created by annealing DNA oligonucleotides 1 and 2. The insert was cloned into *Sac*I and *Xba*I digested pBSII KS+ (Stratagene) to create pBS B4 −59/−1. The plasmid was cut with *Xba*I and transcribed with T7 RNA polymerase. The 81-nucleotide RNA contains 22 nucleotides of polylinker sequence, followed by 59 nucleotides of B4 sequence.

(−59/−31)/(−21/+1) B4/B1 RNA. An insert containing the sequences for this RNA was created by annealing DNA oligonucleotides 3–6. The insert was cloned into *Sac*I and *Xba*I digested pBSII KS+ to create pBSII B4/B1. The plasmid was cut with *Xba*I and transcribed with T7 RNA polymerase. The 73-nucleotide RNA contains 23 nucleotides of polylinker sequence, 29 nucleotides of B4 sequence, and 21 nucleotides of cyclin B1 sequence.

(−62/−22)/(−30/−1) B1/B4 RNA. An insert containing the sequence for this RNA was created using DNA oligonucleotides 7–10. The insert was cloned into *Sac*I and *Xba*I digested pBSII KS+ to create pBSII B4/B1. The plasmid was cut with *Xba*I and transcribed with T7 RNA polymerase. The 92-nucleotide RNA contains 21 nucleotides of polylinker sequence, 41 nucleotides of cyclin B1 sequence, and 30 nucleotides of B4 sequence.

RNA1 (as designated in Figure 8). An insert containing the sequence for this RNA was created using DNA oligonucleotides 11–14. The insert was cloned into *Kpn*I and *Xba*I cut pGEM3Z (Promega) to create pGEM c-*mos* −28/−3. The plasmid was cut with *Xba*I and transcribed with T7 RNA polymerase to give a 93-nucleotide RNA. RNA1 contains 67 nucleotides of polylinker sequence, followed by 26 nucleotides of c-*mos* sequence.

RNA2 (as designated in Figure 8). An insert containing the sequence for this RNA was created using DNA oligonucleotides 15 and 16. The insert was cloned into *Hin*dIII and *Pst*I cut pGEM c-*mos* −28/−3 to create pGEM c-*mos* −28/−3 Æ−23. The plasmid was cut with *Xba*I and transcribed with T7 RNA polymerase to give a 92-nucleotide RNA. RNA2 contains 67 nucleotides of polylinker sequence, followed by 25 nucleotides of c-*mos* sequence.

Preparation of Synthetic mRNAs

CDK1 mRNAs. 5′-terminally capped mRNA encoding *Xenopus* wild-type or K33R CDK1 were prepared from *Bam*HI-cut pET8c-Xlcdc2 and pET8c-Xlcdc2 K33R (Nebreda *et al.*, 1995), using T7 RNA

polymerase (Promega) and ^{7m}GpppG (New England Biolabs). Transcription reactions were performed using 5 μg of template in a 50-μl reaction as described (Melton *et al.*, 1984). mRNA was redissolved in water at a final concentration of 1 mg/ml.

HA-CPEB mRNA. *Xenopus* CPEB cDNA was obtained using a combination of reverse-transcription and polymerase chain reaction. Three hundred nanograms of total *Xenopus* egg RNA was reverse-transcribed using random hexamers and the GeneAmp RNA polymerase chain reaction kit (Perkin Elmer-Cetus). Five microliters of this reaction was then used as template in a 100-μl polymerase chain reaction containing 45 pmol of oligonucleotides 17 and 18 as primers, 200 μ[m] dNTPs, and 2.5 units of *Pfu* DNA polymerase (Stratagene). The reaction was incubated at 95°C for 1 min, 55°C for 1 min, and 72°C for 10 min, and the process was repeated for 25 cycles. The amplified cDNA was digested with *Nde*I and *Sal*I, and subcloned into *Nde*I and *Sal*I digested pAS1/CYH2 (S. Elledge) to give pAS1-CPEB.

pHA-CPEB was prepared by subcloning the *Eco*RI to *Sal*I fragment of pAS1-CPEB into *Eco*RI and *Xho*I digested pGEM7Z (Promega). This fragment contains the full-length CPEB open reading frame (Hake and Richter, 1994), together with the amino terminal HA-epitope, MAYPYDVPDYASLGGH.

5′-terminally capped mRNA encoding HA-tagged CPEB was prepared from *Xba*I-restricted pHA-CPEB, using SP6 RNA polymerase (Promega) and ^{7m}GpppG analogue (New England Biolabs). Transcription reactions were performed using 5 μg of template in a 50-ul reaction as described (Melton *et al.*, 1984). mRNA was resuspended in water at a final concentration of 1 mg/ml.

Oocyte Maturation

Adult female *Xenopus laevis* were primed with 50 units of pregnant mare serum (Sigma) at least 3 days before oocyte isolation. Healthy, stage VI oocytes were manually dissected from excised portions of ovary, and incubated at 18°C in Marc's Modified Ringer's Solution (MMR: 100 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.4, 0.1 mg/ml penicillin and streptomycin). Oocyte maturation was induced by incubation in 10 μg/ml progesterone (Sigma) in MMR, and detected by the appearance of a white spot on the animal pole.

Inhibition of CDK1

To overexpress CDK1 proteins, oocytes were given injections of 50 ng of the either CDK1 wt or CDK1 K33R mRNA, and incubated overnight (~16 h). Half of each of the injected cells were then incubated with progesterone. In some cases, oocytes were injected with 50 nl (5 fmol) of radiolabeled RNA substrates before treatment with progesterone. Oocytes were collected when all of the progesterone-treated, control oocytes had matured. Expression of the K33R CDK1 routinely inhibited progesterone-stimulated maturation by at least 70%, and only inhibited cells were used for further study.

c-mos Oligonucleotides and Prosthetic RNA

DNA oligonucleotide complementary to c-*mos* mRNA at a position 126 nucleotides upstream from the site of poly(A) addition was used to prevent translation of c-*mos* mRNA and block oocyte maturation as described (Sheets *et al.*, 1995). The sense-strand oligonucleotide to the same region was used as a control. Inhibition by the antisense oligo was reversed by injection of 20 ng of a 'prosthetic' RNA, which provides the c-*mos* polyadenylation signals *in trans* (Sheets *et al.*, 1995).

H1-Kinase Assays

Protein kinase activity was measured on clarified extract prepared from groups of five oocytes. Oocytes were homogenized in 10 μl per cell of cold H1 kinase buffer (80 mM sodium β-glycerophosphate, pH 7.4, 20 mM EGTA, and 15 mM $MgCl_2$). Lysates were centrifuged at 10,000 × *g* for 10 min, and 30–35 μl of clarified extract withdrawn with a fine pipet. Histone H1 kinase assays were performed on 5 μl (0.5 cell equivalent) of extract as described (Nebreda *et al.*, 1995). Proteins were separated by 15% SDS-PAGE (Laemmli, 1970), and radioactivity detected by autoradiography.

Immunoblotting

Immunoblots of frog extracts were prepared essentially as described (Ballantyne *et al.*, 1995), except that CDK1 and HA-CPEB proteins were separated by 10% SDS-PAGE. Blots were probed and washed, and the signal detected as described (Ballantyne *et al.*, 1995). Poly(A) polymerase and HA-CPEB were detected using the mouse monoclonal antibodies NN2 (Thuresson *et al.*, 1994) and 12CA5 (Berkeley Antibody Co.) as described (Ballantyne *et al.*, 1995). Blots were probed for CDK1 using a 1:1000 dilution of the monoclonal antibody 3E1 (Nebreda *et al.*, 1995).

Analysis of Injected RNA

Total RNA was isolated from single, injected oocytes as described (Fox *et al.*, 1989), except that proteinase K was omitted from the buffer. Half of each RNA sample (0.5 oocyte equivalents) was then separated on a 6% denaturing polyacrylamide gel (Sanger *et al.*, 1977), and radioactivity was detected by autoradiography with an intensifying screen.

Northern Analysis of Endogenous Cyclin B1 mRNA

Total RNA was isolated from groups of five oocytes as described (Fox *et al.*, 1989), except that proteinase K was omitted from the buffer. RNA was separated on a 0.8% formaldehyde-agarose gel, and transferred to BioTrans nylon membrane with 10× standard saline-citrate (SSC). Northern blots were prehybridized for 4 h at 65°C in 10 ml of hybridization solution (5× SSC, 5× Denhardt's, 10 mM EDTA, 1% SDS, 0.1 mg/ml yeast tRNA, 0.1 mg/ml poly(rA), and 10% dextran sulfate) containing 0.1 mg/ml of boiled, sheared salmon sperm DNA. A single-stranded RNA hybridization probe to cyclin B1 was prepared by in vitro transcription of *Eco*RI cut pGEM −83/+2 cyclin B1 (Sheets *et al.*, 1994) with SP6 RNA polymerase (Promega) as described (Melton *et al.*, 1984). RNA was prepared for hybridization by boiling for 1 min with 100 μg of salmon sperm DNA. Hybridization was carried out for greater then 14 h at 65°C in 10 ml of fresh hybridization solution to which prepared probe was added. Blots were washed for 30 min at 65°C with solutions of 2× SSC, 0.1% sodium dodecyl sulfate (SDS), and 0.5× SSC, 0.1% SDS, respectively. Radioactivity was detected by autoradiography with an intensifying screen.

Metabolic Labeling and Immunoprecipitation

Oocytes were microinjected with ~50 nl (100 ng) of antisense or sense c-*mos* oligonucleotide, and incubated for 1 h in MMR. Cells were then administered microinjections of ~50 ng of HA-CPEB mRNA and incubated overnight at 18°C in MMR containing 2.5 mCi/ml of [^{32}P]orthophosphate (Amersham) and 0.5 mCi/ml [^{35}S]methionine/cysteine (Translabel, Amersham). Ten oocytes were placed in each labeling solution, either containing or lacking progesterone. Cells were collected 12 h later, at which time all sense oligonucleotide-injected, progesterone-treated oocytes had undergone maturation. Cells were washed three times in MMR, and homogenized in 400 μl of lysis buffer (10 m[m] sodium phosphate, pH 7.2, 150 m[m] NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS, 80 m[m] β-glycerophosphate, 50 m[m] NaF, 20 m[m] EGTA, and 10 μg/ml chymostatin, pepstatin, and leupeptin). Lysates were centrifuged at 10,000 × *g* for 10 min at 4°C, and the labeled supernatants immunoprecipitated with the 12CA5 monoclonal antibody as described (Ballantyne *et al.*, 1995). Radiolabeled proteins (5 cell equivalents) were separated by 10%

SDS-PAGE, and visualized by autoradiography. ^{32}P was distinguished from total radiolabel based on penetration through four sheets of x-ray film (KODAK X-OMAT AR5).

Cycloheximide Inhibition of Protein Synthesis

Oocytes were incubated in 15 μg/ml cycloheximide in MMR for 2 h before either injection or incubation in [^{35}S]methionine and [^{35}S]cysteine (50 μCi/ml). Cells were then incubated for 12 h at 18°C. Incorporation of ^{35}S into acid-precipitable form was used to monitor the effect of cycloheximide on total cellular protein syntesis. ^{35}S-labeled cells were rinsed three times in MMR, homogenized in 50 μL/cell of lysis buffer, and centirufuged at 10,000 × *g* for 10 min at 4°C. 250 μl of 1 N NaOH was added to 5 μl of labeled supernatant, and incubated at 37°C for 10 min. One milliliter of 25% TCA containing 2% casamino acids was added, and the mixture was incubated on ice for 30 min. Precipitated proteins were collected on Whatman GF/A glass fiber filters. Filters were washed 3 times with 5% TCA, 1 time with acetone, and dried for 30 min. Radioactivity on the filters was determined by liquid scintillation counting.

RESULTS

CDK1-Independent Activation of c-mos Polyadenylation

To determine whether activation of CDK1 is required for cytoplasmic polyadenylation, we exploited a mutant form of CDK1 kinase. The mutant protein, K33R CDK1, contains arginine instead of lysine at position 33 within the ATP binding site (Pickham *et al.*, 1992). It lacks kinase activity, but can still associate with cyclins; it thus blocks oocyte maturation by sequestering cyclins into unproductive complexes (Solomon *et al.*, 1992; Furuno *et al.*, 1994; Nebreda *et al.*, 1995).

We tested whether oocytes expressing K33R CDK1 could polyadenylate c-*mos* RNA in response to progesterone. To do so, oocytes first were given injections of mRNA encoding wild-type or K33R forms of CDK1. After incubation, to allow accumulation of protein translated from the injected mRNA, the oocytes were given injections of a ^{32}P-labeled, synthetic form of the c-*mos* 3′-UTR. Progesterone was then added, and polyadenylation was assayed by gel electrophoresis and autoradiography.

The wild-type and K33R CDK1 mRNAs increased the level of CDK1 protein five- to sevenfold relative to that in cells that did not receive injections, as judged by immunoblotting with anti-CDK1 antibody (Figure 1A). As expected (Nebreda *et al.*, 1995), the mutant, but not the wild-type, protein prevented activation of endogenous CDK1 in response to progesterone as monitored by an increase in H1 kinase activity (Figure 1B), and blocked maturation. Despite their cell cycle arrest, progesterone-treated oocytes overexpressing K33R CDK1 polyadenylated c-*mos* RNA efficiently (Figure 1C, lanes 3 and 4). The extent of polyadenylation was comparable to that in oocytes that were not overexpressing any form of CDK1 (Figure 1C, lanes 1 and 2), or were overexpressing wild-type CDK1 (Figure 1C, lanes 5 and 6). We conclude that the appearance of c-*mos* polyadenylation activity in response to progesterone can be

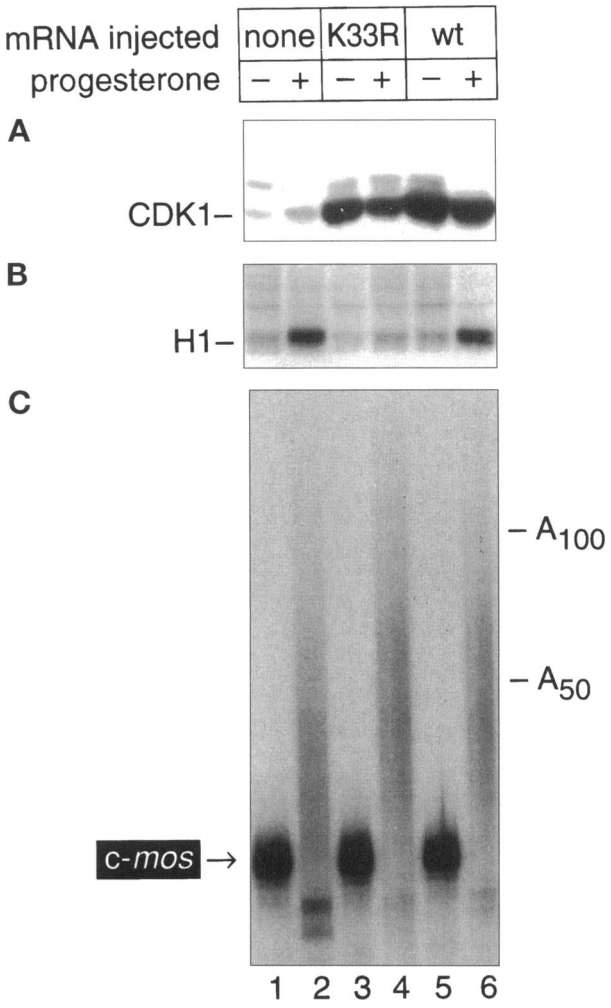

Figure 1. CDK1-independent cytoplasmic polyadenylation. (A) Immunoblot showing total amount of CDK1 protein present in oocytes given injections of CDK1 mRNAs. Approximately 10 μg of protein, corresponding to 0.5 oocyte, were analyzed in each lane. Lanes 1 and 2, no mRNA. Lanes 3 and 4, CDK1 K33R mRNA (50 ng injected). Lanes 5 and 6, CDK1 mRNA (50 ng injected). Oocytes were incubated with (even lanes) or without (odd lanes) progesterone. (B) Histone H1 kinase activity present in extracts prepared from oocytes treated as in (A). (C) Polyadenylation of c-*mos* RNA does not require activation of CDK1. Electrophoretic mobility of an injected, ^{32}P-labeled c-*mos* polyadenylation substrate recovered from oocytes given injections of CDK1 mRNAs. Lanes 1 and 2, no mRNA. Lanes 3 and 4, CDK1 K33R mRNA (50 ng injected). Lanes 5 and 6, CDK1 mRNA (50 ng injected). Oocytes were incubated with (even lanes) or without (odd lanes) progesterone. Each lane contains RNA isolated from a single oocyte. Similar results were obtained in three separate experiments, using different frogs. Lengths of poly(A) added are indicated to the right of the panel. The position of the injected RNA is indicated to the left.

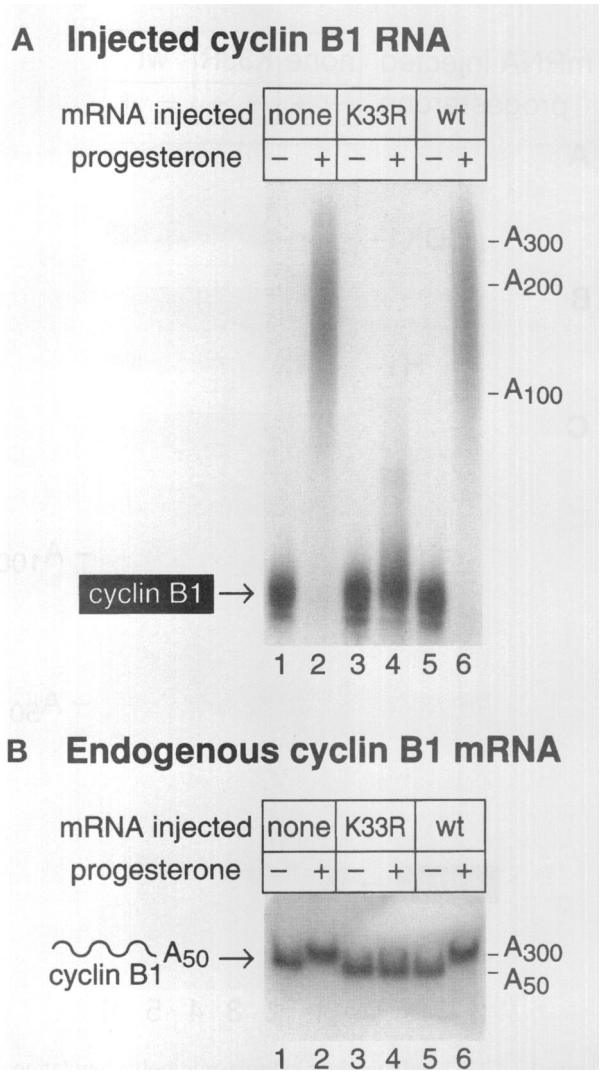

Figure 2. CDK1-dependent cytoplasmic polyadenylation. (A) Polyadenylation of cyclin B1 RNA requires activation of CDK1. Electrophoretic mobility of an injected, ^{32}P-labeled cyclin B1 RNA recovered from oocytes injected with CDK1. These data were obtained using the same group of oocytes as shown in Figure 1. Lanes 1 and 2, no mRNA; Lanes 3 and 4, CDK1 K33R mRNA (50 ng injected); Lanes 5 and 6, CDK1 mRNA (50 ng injected). Oocytes were incubated with (even lanes) or without (odd lanes) progesterone. Each lane contains RNA isolated from a single oocyte. Similar results were obtained in three separate experiments, using different frogs. Lengths of poly(A) added are indicated to the right of the panel. The position of the injected RNA is indicated to the left. (B) Polyadenylation of endogenous cyclin B1 mRNA requires activation of CDK1. Northern blot showing mobility of endogenous cyclin B1 mRNA present in oocytes treated as above. Each lane contains one-half of the total RNA isolated from 10 oocytes (i.e., 5 oocyte equivalents). Similar results were obtained in two separate experiments, using different frogs. The positions of cyclin mRNAs bearing 50- and 300-nucleotide poly(A) tails are indicated to the right.

uncoupled in vivo from activation of CDK1, MPF, and progression through oocyte maturation.

Polyadenylation of Cyclin B1 RNA Is Blocked by K33R CDK1

To test whether polyadenylation of cyclin B1 RNA was independent of CDK1, we repeated the analysis above using a synthetic form of the cyclin B1 3′-UTR. In contrast to c-*mos* RNA, polyadenylation of injected cyclin B1 RNA was dramatically impaired in oocytes expressing K33R (Figure 2A).

To confirm that polyadenylation of cyclin B1 mRNA was inhibited by K33R CDK1, we examined endogenous cyclin B1 mRNA by Northern blotting (Figure 2B). The long (ca 300 nucleotides) poly(A) tail added to cyclin B1 mRNA during oocyte maturation can be detected by a reduction in the electrophoretic mobility of the mRNA (Figure 2B, compare lanes 1 and 2). This polyadenylation reaction is blocked by overexpression of K33R CDK1 (lanes 3 and 4) but occurs normally in oocytes overexpressing the wild-type form of CDK1 (lanes 5 and 6). We conclude that cytoplasmic polyadenylation of cyclin B1 mRNA, unlike that of c-*mos* RNA, is dependent on activation of CDK1.

c-mos-Dependent and c-mos-Independent Modes of Activation

Removal of the polyadenylation signals from endogenous c-*mos* mRNA using targeted antisense oligonucleotides and RNAse H prevents the increase in c-*mos* protein levels and activation of MPF after progesterone treatment (Sheets *et al.*, 1995; Barkoff *et al.*, manuscript in preparation). Because amputation of c-*mos* mRNA prevents activation of CDK1, and CDK1 seems to be required for polyadenylation of cyclin B1 mRNA (Figure 2), we predicted that c-*mos* polyadenylation should be required for polyadenylation of cyclin B1 mRNA. To test this prediction, we injected a cyclin B1 RNA substrate into oocytes that previously had been given injections of an anti-c-*mos* oligonucleotide (Figure 3). This oligonucleotide, complementary to a region 126 nucleotides from the 3′ end of c-mos mRNA's 3′-UTR, causes digestion of the RNA strand by endogenous RNAse H. The amputated c-*mos* mRNA that results cannot be polyadenylated (Sheets *et al.*, 1995). As shown in Figure 3A (lanes 1–4), cyclin B1 polyadenylation was impaired dramatically by injection of the anti-c-*mos* oligonucleotide. Injection of the control sense-strand oligonucleotide had no effect (Figure 3A, lanes 5 and 6). In contrast, injection of the anti-c-*mos* oligonucleotide had little effect on polyadenylation of injected c-*mos* RNA substrate (Figure 3B). (The injected RNA is not digested by RNaseH because it lacks the region complementary to the DNA oligonucleotide.) The poly(A) tails added to c-*mos* RNA in the antisense injected cells in fact are slightly longer, on average, than those added in uninjected cells, or in

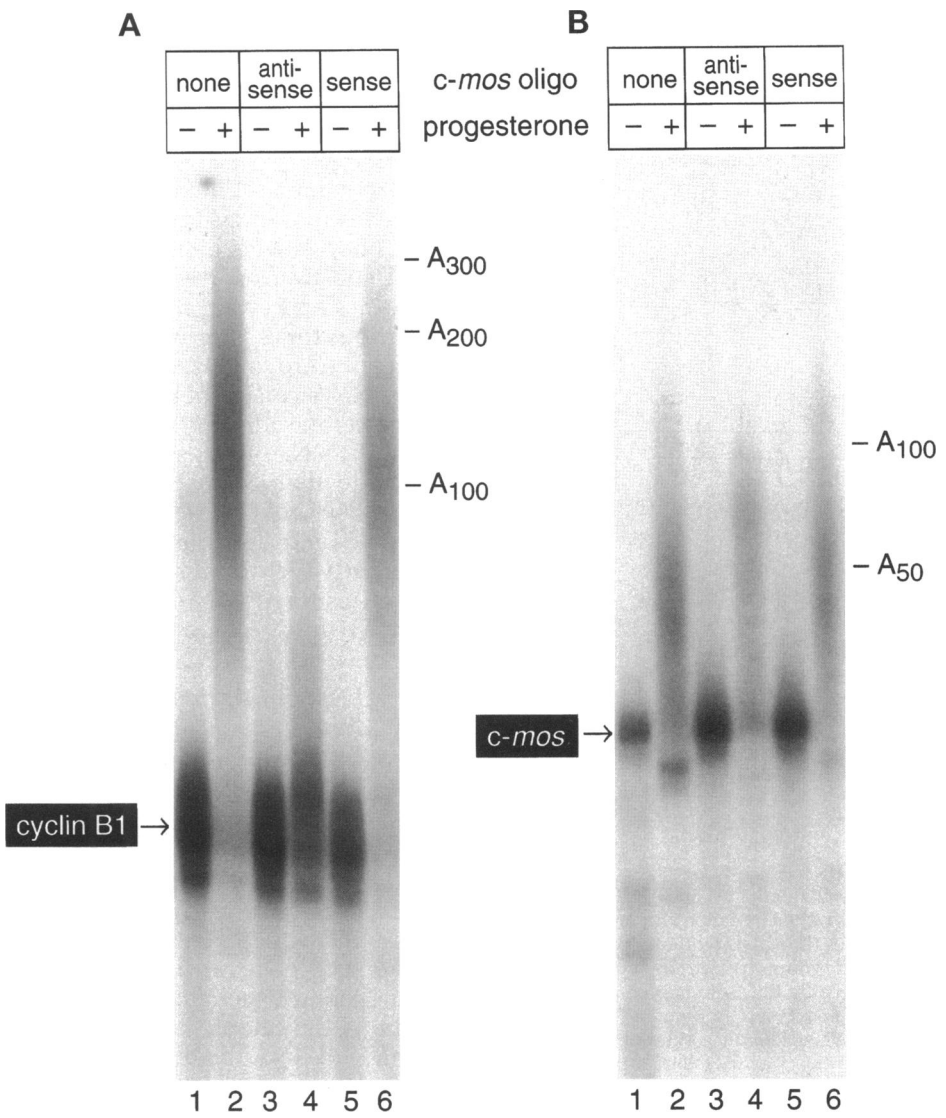

Figure 3. Differing requirements for c-*mos* protein kinase defines two classes of cytoplasmic polyadenylation. (A) Mobility of an injected cyclin B1 RNA recovered from oocytes given injections of various DNA oligonucleotides. Lanes 1 and 2, no oligonucleotide. Lanes 3 and 4, antisense c-*mos* oligonucleotide complementary to region −126 nucleotide from site of poly(A) addition. Lanes 5 and 6, sense-strand of c-*mos* oligonucleotide. Oocytes were incubated with (even lanes) or without (odd lanes) progesterone. (B) Mobility of injected c-*mos* RNA recovered from oocytes treated as in (A). Lanes are designated as in (A). In both (A) and (B), each lane contains RNA isolated from a single oocyte. Similar results were obtained in four separate experiments, using different frogs. Positions of RNAs bearing different lengths of poly(A) are indicated to the right of the panel. Positions of the injected RNAs are indicated to the left.

cells injected with the sense-strand oligonucleotide. This may be a result of the lack of nuclear envelope breakdown in the antisense-injected cells, because nuclear breakdown is required for activation of a maturation-specific deadenylase (Varnum *et al.*, 1992).

To corroborate these results obtained with injected RNAs, we examined polyadenylation of endogenous cyclin B1 mRNA by Northern blotting (Figure 4). As expected, endogenous cyclin B1 mRNA was not polyadenylated in oocytes given injections of the antisense c-*mos* oligonucleotide (Figure 4B, lanes 1–4). To demonstrate directly that polyadenylation of cyclin B1 mRNA requires polyadenylation of c-mos mRNA, we used a "prosthetic RNA" strategy, in which c-*mos* polyadenylation signals were restored to amputated c-mos mRNA by base-pairing to an injected prosthetic RNA, as diagrammed in Figure 4A. This prosthetic RNA rescues maturation, c-*mos* translation, and MPF levels in

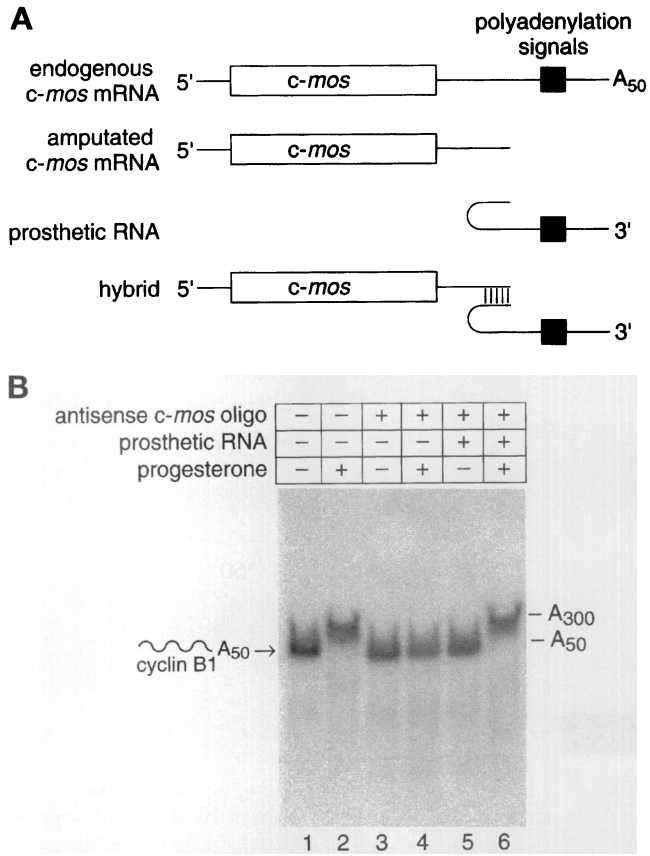

Figure 4. Cytoplasmic polyadenylation of cyclin B1 mRNA requires polyadenylation of c-*mos* mRNA. (A) Amputation and prosthetic RNA strategy. The black box designates the sgnals required for polyadenylation of c-mos mRNA, contained within the last 126 nucleotides of its 3′-UTR. These signals are removed by RNAse digestion after injection of an antisense oligonculeotide, generating amputated c-mos mRNA. The prosthetic RNA contains the last 126 nucleotides of c-*mos* mRNA and a region complementary to the 3′ end of the amputated c-mos mRNA. Upon injection into an oocyte containing amputated c-*mos* mRNA, the prosthetic RNA hybridizes to the amputated mRNA, restoring its translation, activation of MPF, and rescuing oocyte maturation (Sheets *et al.*, 1995; Barkoff *et al.*, manuscript in preparation). (B) Northern blot showing the mobility of cyclin B1 mRNA recovered from oocytes given injections of antisense c-*mos* oligonucleotides and prosthetic RNA. Oocytes were incubated with (even lanes) or without (odd lanes) progesterone. Oocytes were given injections of the prosthetic RNA 1 h after injection of the oligonucleotides. Lanes 1 and 2, no c-*mos* oligonucleotide; lanes 3 and 4, antisense c-*mos* oligonucleotide; lanes 5 and 6, antisense c-*mos* oligonucleotide and prosthetic RNA. Each lane contains one-half of the total RNA isolated from 10 oocytes (i.e., 5 oocyte equivalents). Similar results were obtained in three separate experiments, using different frogs. The positions of cyclin mRNAs bearing 50- and 300-nucleotide poly(A) tails are indicated to the right.

oocytes containing amputated c-*mos* mRNA (Sheets *et al.*, 1995; Barkoff *et al.*, manuscript in preparation). It is striking that the same prosthetic RNA, carrying c-*mos* signals and targeted to c-*mos* mRNA, rescues polyadenylation of cyclin B1 mRNA (Figure 4B, lanes 5 and 6). We conclude that polyadenylation of c-*mos* mRNA is required for polyadenylation of cyclin B1 mRNA.

c-mos RNA Is Polyadenylated before Cyclin B1 RNA

The above model predicts that c-*mos* polyadenylation precedes that of cyclin B1. To test whether this is the case, oocytes were given injections of synthetic labeled RNA substrates corresponding to terminal regions of the c-*mos* and cyclin B1 3′-UTRs. Cells were collected at various times after progesterone addition, and RNA samples were analyzed. Whereas polyadenylation of c-*mos* RNA can be detected as early as 2 hours after progesterone addition (Figure 5A), polyadenylation of cyclin B1 RNA is not seen until 4–7 h (Figure 5B). We conclude that c-*mos* RNA is polyadenylated before cyclin B1 RNA. These data, combined with those in Figure 3 and 4, strongly suggest that the temporal order in which c-*mos* and cyclin B1 mRNAs receive poly(A) is imposed, at least in part, by dependence of cyclin B1 mRNA's polyadenylation on that of c-*mos*.

Two Classes of Activation and mRNA

We have assessed whether the polyadenylation behavior of either c-*mos* or cyclin B1 RNA is unique by injecting four additional RNA substrates. These were derived from the 3′ UTRs of mRNAs encoding cyclin A1 (Kobayashi *et al.*, 1991), the histone-related protein B4 (Smith *et al.*, 1988), the putative transcription factor D7 (Smith *et al.*, 1988), and a protein of unknown function (H4; Dworkin and Dworkin, 1985). Each RNA receives poly(A) during frog oocyte maturation. Preventing maturation by injection of the antisense c-*mos* oligonucleotide did not block the cytoplasmic polyadenylation of cyclin A1, B4, and H4 RNA (Figure 6, A–C). In contrast, progesterone-induced polyadenylation of D7 RNA was prevented in oocytes given injections of antisense c-*mos* oligonucleotide (Figure 6D).

We conclude that two classes of mRNA and of cytoplasmic polyadenylation exist during oocyte maturation. Class I polyadenylation, exemplified by c-*mos*, cyclin A1, B4, and H4 RNAs, is c-*mos* independent. Class II polyadenylation, exemplified by cyclin B1 and D7 RNAs, requires prior activation of the class I system, and polyadenylation of c-*mos* mRNA in particular. We refer to both the polyadenylation events, and to the mRNAs, as classes I and II. The two classes of polyadenylation may differ in positive-acting factors, repressors, or both.

Sequences that Specify Class I versus Class II

To define sequences that are required to specify class I versus class II behavior, we prepared chimeric RNAs between the 3′-UTRs of mRNAs encoding cyclin B1 (class II) and the histone-like protein B4 (class I). The sequences of the relevant portions of the chimeric RNAs

are depicted in Figure 7. In the chimeric RNAs, the class of polyadenylation was specified by sequences upstream of AAUAAA. Thus, a chimeric RNA possessing cyclin B1 sequences upstream of AAUAAA, with B4 sequences downstream (B1/B4), behaved very similarly to cyclin B1 RNA; injection of anti-c-*mos* oligonucleotides reduced its polyadenylation (Figure 7, lanes 1–4), as it did that of cyclin B1 RNA (lanes 5–8). The opposite chimeric RNA, in which B4 sequences are upstream, and cyclin B1 sequences downstream, behaved like B4 RNA. Polyadenylation of the B4/B1 chimera was not inhibited by amputation of c-*mos* mRNA (lanes 9–12); the behavior of this RNA was very similar to that of B4 RNA (lanes 13–16).

These experiments indicate that the sequences that specify class I versus class II lie upstream of AAUAAA. Because the upstream sequences include CPEs, we next determined whether a CPE is sufficient to distinguish the two classes. To do so, we first inserted the sequence UUUUUAU, a canonical CPE, into an RNA that otherwise would not receive poly(A) during oocyte maturation, the 3′-UTR of SV40 late mRNAs. The new RNA, designated SV40 + CPE, now received poly(A) during maturation via the class I pathway, in that its polyadenylation was unaffected by injection of the antisense c-*mos* oligonucleotide (Figure 8A). Similarly, insertion of UUUUUAU into a second "naive" RNA, one corresponding to the 3′-UTR of ribosomal protein L1 mRNA, also caused class I polyadenylation during maturation (unpublished observations). We conclude that the CPE, UUUUUAU, in the absence of other sequence information, promotes class I polyadenylation.

To test whether the character of a CPE is sufficient to dictate class I versus class II polyadenylation, we compared the polyadenylation of two RNAs that are identical except for a single nucleotide change in the CPE. These RNAs are derived from the 3′ terminal portion of the c-*mos* 3′-UTR. RNA1 carries the CPE, UUUUAU, found in c-*mos* mRNA adjacent to AAUAAA. Its polyadenylation is not inhibited by injection of anti-c-*mos* oligonucleotide (Figure 8B, lanes 1–4). In RNA2, the UUUUAU has been changed to UUUUU through the deletion of a single adenosine. Polyadenylation of RNA2 is impaired, but not abolished, by amputation of c-*mos* mRNA (Figure 8B, lanes 5–8). These data suggest that the precise sequence of the CPE plays an important role in determining class I versus class II behavior.

Activation of Class I Polyadenylation Is Independent of Detectable CPEB and PAP Phosphorylation, and Requires Cellular Protein Synthesis

Several *trans*-acting factors have been implicated in polyadenylation during frog oocyte maturation. These include poly(A) polymerase (PAP) and CPE-Binding Protein (CPEB; Hake and Richter, 1994), both of which are phosphorylated during oocyte maturation (Paris *et al.*, 1991; Ballantyne *et al.*, 1995). Previous work suggested that phosphorylation of CPEB was critical in the activation of cytoplasmic polyadenylation (Paris *et al.*, 1991). To test the role of these phosphorylation reactions in class I polyadenylation, we determined whether either CPEB or PAP were phosphorylated in progesterone-treated oocytes arrested by amputation of c-*mos* mRNA. Any event required for class I polyadenylation must, by definition, occur in such cells.

Progesterone-stimulated phosphorylation of endogenous PAP results in a change in electrophoretic mobility that can be detected by immunoblotting (Ballantyne *et al.*, 1995). Multiple forms of the protein exist in the resting oocyte prior to progesterone treatment, and differ in phosphorylation state (Ballantyne *et al.*, 1995). As shown in Figure 9A, progesterone-induced hyperphosphorylation of PAP is not detected in oocytes given injections of an antisense c-*mos* oligonucleotide (Figure 9A, lanes 1 and 2), but occurs normally in oocytes injected with the control, sense-strand oligonucleotide (Figure 9A, lanes 3 and 4). Similarly, overexpression of K33R CDK1 blocks hyperphosphorylation of PAP (unpublished observations). We conclude that progesterone-induced hyperphosphorylation of PAP is not required for activation of class I polyadenylation.

To examine the possible role of CPEB phosphorylation, we utilized epitope-tagged CPEB protein (HA-CPEB). HA-CPEB was expressed in oocytes by injection of synthetic mRNA. After injection, oocytes were incubated simultaneously with [^{35}S]methionine/cysteine and [^{32}P]orthophosphate, and then treated with progesterone. The phosphorylation state and expression of HA-CPEB was monitored by immunoprecipitation with an antibody directed against the HA epitope. Filters were used to distinguish ^{32}P from total radioactivity. Figure 9B shows the signal derived from ^{32}P, whereas Figure 9C shows signal from ^{35}S as well.

HA-CPEB recovered from progesterone-treated oocytes that had been given injections of a control sense-strand oligonucleotide, contained ^{32}P; the same oocytes not treated with progesterone did not yield significant ^{32}P-HA-CPEB (Figure 9B, lanes 3 and 4). These data demonstrate that HA-CPEB is phosphorylated during maturation, as is the endogenous protein (Paris *et al.*, 1991). Injection of anti-c-*mos* oligonucleotides prevented incorporation of ^{32}P into HA-CPEB during maturation (Figure 9B, lanes 1 and 2). Comparable amounts of ^{35}S-labeled HA-CPEB were recovered from each collection of cells, as expected (Figure

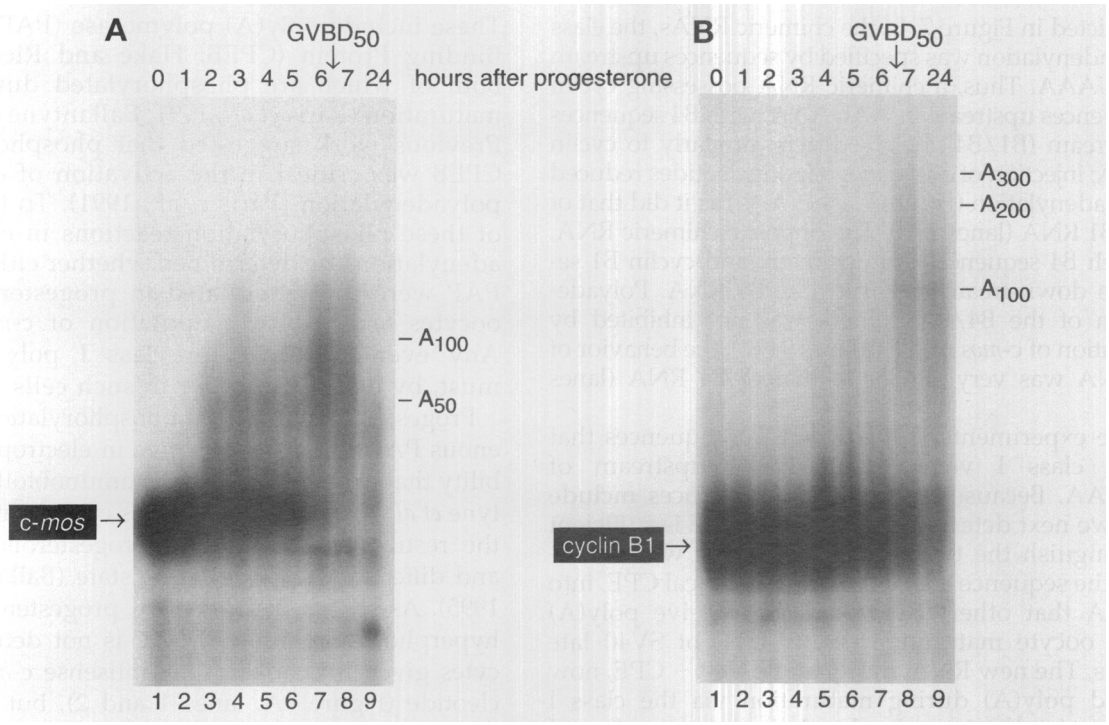

Figure 5. Cytoplasmic polyadenylation of c-*mos* RNA precedes that of cyclin B1. Oocytes were given injections of 5 fmol of ^{32}P-labeled RNA derived from either c-*mos* or cyclin B1 mRNA. Oocytes were incubated in progesterone, groups of 10 cells collected at the times indicated, and maturation scored by the presence of white spot on the animal pole. RNA was isolated and analyzed by denaturing polyacrylamide electrophoresis. The times after progesterone addition are indicated above each lane. $GVBD_{50}$ indicates the time at which 50% of the oocytes had undergone germinal vesivle breakdown, indicative of the completion of first meiosis. (A) c-*mos* RNA. (B) cyclin B1 RNA. In both (A) and (B), each lane contains one oocyte equiavlent of RNA. Similar results were obtained in two separate experiments, using different frogs. Positions of RNAs bearing bearing different lengths of poly(A) are indicated to the right of the panel. Positions of the injected RNAs are indicated to the left.

9C, lanes 1–4). We conclude that class I polyadenylation is independent of detectable CPEB phosphorylation.

To test whether activation of class I polyadenylation requires protein synthesis, oocytes were incubated in cycloheximide, and then given injections of labeled c-*mos* RNA. Cycloheximide inhibited incorporation of [^{35}S]methionine and [^{35}S]cysteine by 95%. In response to progesterone, cycloheximide-treated cells neither matured (Wasserman and Masui, 1975) nor polyadenylated the injected c-*mos* RNA (Figure 10). Thus, activation of c-*mos* polyadenylation likely requires either continued synthesis of a protein that was present before maturation, or translation of a new protein that is induced by progesterone.

DISCUSSION

Our findings suggest a dependent pathway comprising two classes of cytoplasmic polyadenylation reactions. Class I polyadenylation is activated independent of c-*mos* stimulation, and is insensitive to inhibition by the dominant negative (K33R) form of CDK1. In contrast, class II polyadenylation requires polyadenylation of c-*mos* mRNA, and is prevented by the mutant CDK1. Class II reactions require activation of class I polyadenylation, and of c-*mos* mRNA in particular. This pathway imposes a temporal order on polyadenylation of different mRNAs. Consistent with this view, cyclin B1 (Figure 5) and D7 (Fox *et al.*, 1989; data not shown) RNAs, both of which are class II, receive poly(A) after c-*mos* mRNA.

A parsimonious interpretation of the roles of c-*mos* and CDK1 is depicted in Figure 11. Polyadenylation of c-*mos* mRNA increases c-*mos* protein levels, which leads to an increase in MPF (CDK1/cyclin) activity. The mechanism of MPF activation by c-*mos*, studied and discussed by others (reviewed in Sagata, 1997), is not depicted. In the pathway (Figure 11), the K33R form of CDK1 inhibits because it blocks activation of MPF. It could do so directly, or by inhibiting an upstream event in the pathway, as suggested by Nebreda *et al.* (1995). The essential feature of the model are that class I polyadenylation, because of its role in c-*mos* polyadenylation, is required for class II events. In this manner, cytoplasmic polyadenylation of distinct

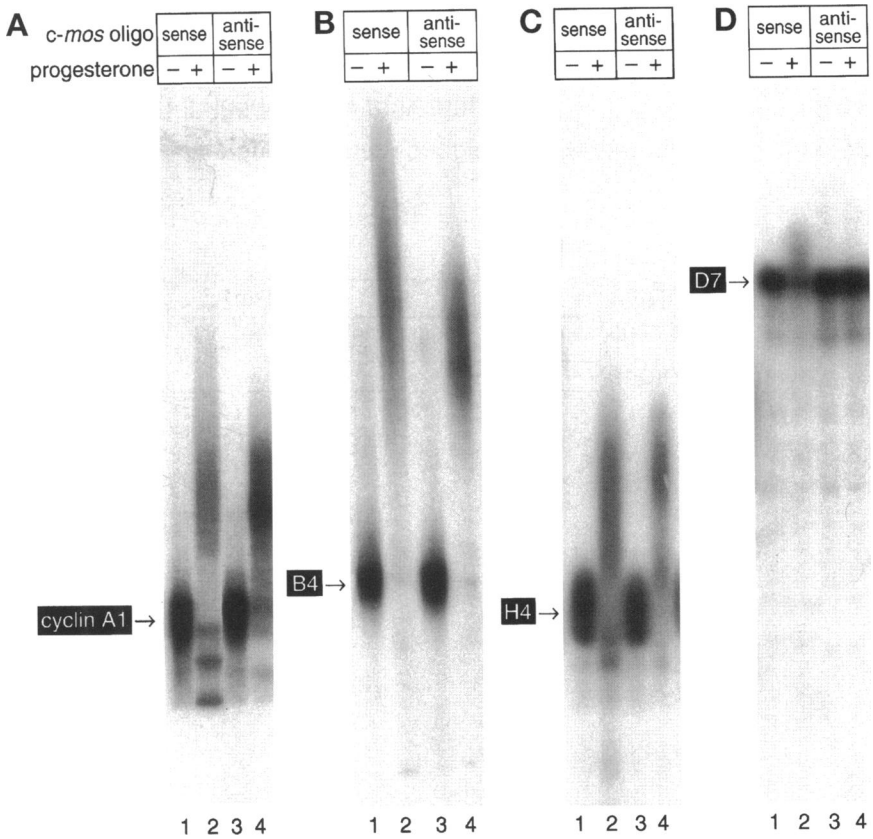

Figure 6. Multiple substrates for class I and class II polyadenylation. Polyadenylation of injected, ^{32}P-labeled RNAs dervied from the 3′ termini of cyclin A1 (A), B4 (B), H4 (C), and D7 (D) mRNAs, as indicated to the left of each panel. To distinguish class I and class II polyadenylation, antisense c-mos oligonucleotides were injected as in Figure 3. Sense-strand oligonucleotides (complementary sequence of the same oligonucleotide) were used as controls. Lane 1, sense oligonucleotide, no progesterone; lane 2, sense oligonucleotide, progesterone treated; lane 3, antisense oligonucleotide, no progesterone; lane 4, antisense oligonucleotide, progesterone treated. Each lane contains RNA isolated from a single oocyte. Similar results were obtained in two separate experiments, using different frogs.

groups of mRNAs become linked to activation of MPF and cell cycle control.

The polyadenylation properties of mRNAs within a class may differ. For example, class I mRNAs may exist that receive poly(A) late in maturation because of sequence-specific repression. Our model predicts, however, that polyadenylation of class II RNAs cannot precede that of c-*mos* mRNA in particular.

MPF is not required to activate cytoplasmic polyadenylation. We prevented MPF activation in vivo by two independent means: by amputation of polyadenylation signals from c-*mos* mRNA, and by overexpression of the K33R form of CDK1. In both cases, class I polyadenylation is unperturbed. These data are consistent with the observation that certain mRNAs receive poly(A) early in maturation, likely before the time that MPF activity increases (McGrew and Richter, 1990; Paris and Richter, 1990; Sheets *et al.*, 1994). In contrast, activation of class II reactions may require MPF activity.

Overexpression of MPF, achieved by injection of cyclin mRNA or protein, causes maturation in the absence of progesterone (Swenson *et al.*, 1986; Pines and Hunt, 1987; Westendorf *et al.*, 1989; Pines and Hunter, 1990; Whitfield *et al.*, 1990). Moreover, it induces polyadenylation of class I mRNAs in particular (Paris *et al.*, 1991; unpublished results). If MPF activation lies downstream of class I polyadenylation, as depicted in Figure 11, how does overexpression of MPF induce c-*mos* polyadenylation? In principle, MPF could be part of a positive feedback loop, in which certain mRNAs (e.g., c-*mos*) are polyadenylated through two distinct modes, before and after first meiosis. This proposal arises in part from the observation that continued c-*mos* translation is required for second meiosis (Daar *et al.*, 1991; Kanki and Donoghue, 1991). A second burst of c-*mos* polyadenylation might be required to sustain translation of c-*mos* mRNA once deadenylation has been triggered by nuclear breakdown at the end of first meiosis (Varnum *et*

B1 ...uuuuacugguuuuAAUAAAgcucauuuuaacaug$_{OH}$

B4 ...GUUUAAUUCUAUAAAUAAAGUAAGUAAUUGUUUUUUUUUUUCUU$_{OH}$

B1/B4 ...uuuacugguuuu/AAUAAAGUAAGUAAUUGUUUUUUUUUUUCUU$_{OH}$

B4/B1 ...UUUAAUUCUAUA/AAUAAAgcucauuuuaacaugu$_{OH}$

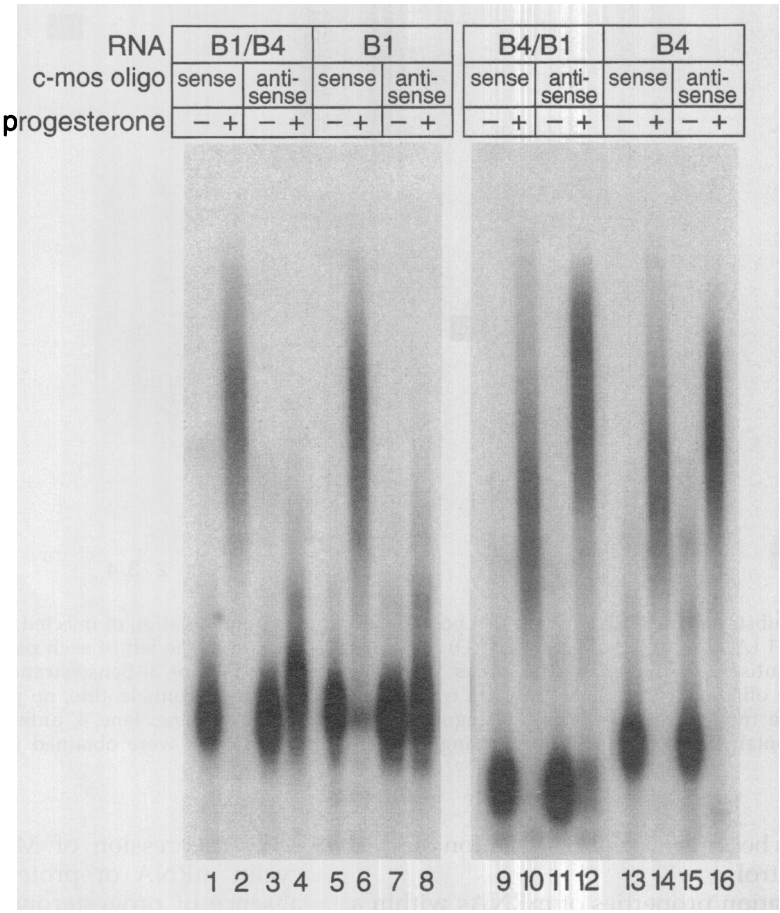

Figure 7. Sequences responsible for distinguishing class I and II polyadenylation reside upstream of the nuclear polyadenylation signal AAUAAA. Chimeric RNAs were prepared between the class II RNA, cyclin B1(B1), and the class I RNA B4 (B4). Two chimeric RNAs were prepared by fusing sequences from each parent RNA at a site immediately upstream of AAUAAA. The sequences of the parental (B1 and B4) and chimeric (B1/B4 and B4/B1) RNAs are provided at the top of the figure, aligned at AAUAAA. Thus, (B1/B4) contains 5′ sequences from cyclin B1, and 3′ sequences (including AAUAAA) from B4. Likewise, (B4/B1) is composed of 5′ B4 sequences and 3′ sequences from cyclin B1. Oligonucleotide complementary to c-*mos* mRNA was used to distinguish class I and class II polyadenylation, as in Figure 3. Lanes 1, 5, 9, 13, sense oligonucleotide, no progesterone; lanes 2, 6, 10, 14, sense oligonucleotide, progesterone-treated; lanes 3, 7, 11, 15, antisense oligonucleotide, no progesterone; lanes 4, 8, 12, 16, antisense oligonucleotide, progesterone-treated. B1/B4 (lanes 1–4), B1 (lanes 5–8), B4/B1 (lanes 9–12), and B4 (lanes 13–16). The behavior of each chimeric RNA reflected the behavior of the RNA from which its 5′ sequences were derived. Each lane contains RNA isolated from a single oocyte. Similar results were obtained in two separate experiments, using different frogs.

al., 1992). Indeed, c-*mos* protein levels increase modestly early in maturation, and then are elevated subsequently more dramatically at nuclear breakdown (Gotoh *et al.*, 1995). A positive feedback loop regulating c-*mos* protein levels also has been proposed based on studies of MAP kinase, and may require activation of MPF (reviewed in Sagata, 1997). Alternatively, overexpression of MPF might activate c-*mos* polyadenylation through mechanisms that are not physiologically relevant.

In defining two classes of polyadenylation, we do not imply that the two classes necessarily differ in the

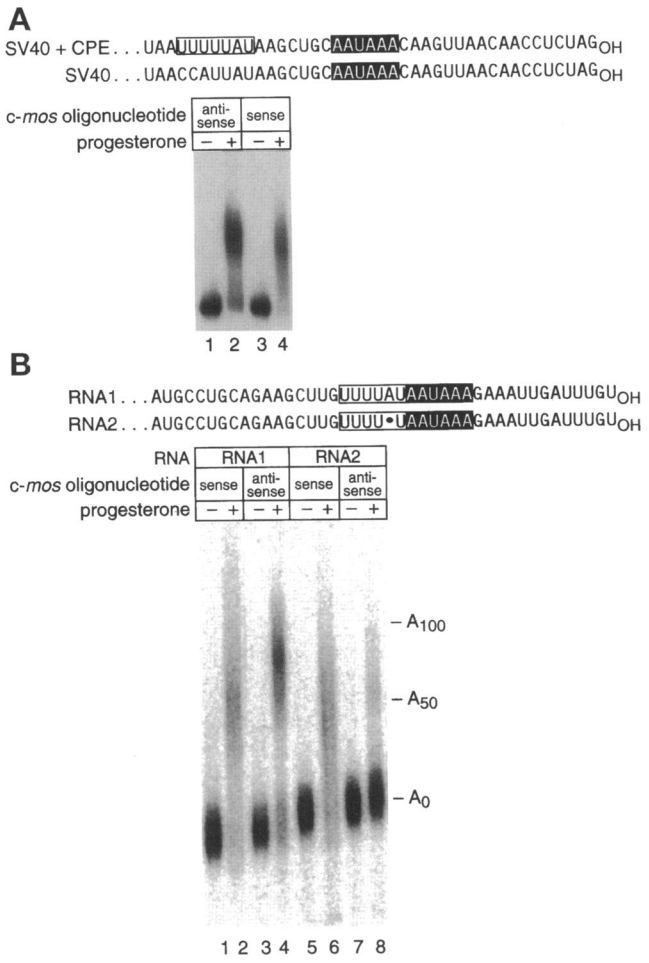

Figure 8. Role of CPE sequences in distinguishing class I and II polyadenylation. (A) The CPE, UUUUUAU, promotes class I polyadenylation. Polyadenylation of a naive RNA (SV40), which has been altered to contain the CPE UUUUUAU (SV40+CPE). The sequence of the relevant version of SV40 and of SV40+CPE RNAs is provided, with the inserted CPE sequence in a box, upstream of AAUAAA. Injection of an oligonucleotide complementary to c-*mos* mRNA was used to distinguish class I and class II polyadenylation as in Figure 3, with a sense-strand oligonucleotide as a control. Lane 1, antisense oligonucleotide, no progesterone; lane 2, antisense oligonucleotide, progesterone treated; lane 3, sense oligonucleotide, no progesterone; and lane 4, sense oligonucleotide, progesterone treated. (B) Subtle changes in the CPE can alter class I versus class II behavior. A comparison of two RNAs differing only in their CPE sequences (immediately upstream of AAUAAA) is shown. The CPE in RNA1 is UUUUAU. In RNA2, the adenosine is deleted, leaving UUUUU. The RNAs are derived from the 3′ end of c-*mos* mRNA. Oligonucleotide complementary to c-*mos* mRNA was used to distinguish class I and class II polyadenylation as above, RNA1 (lanes 1–4), and RNA2 (lanes 5–8). Lanes 1 and 5, sense oligonucleotide, no progesterone; lanes 2 and 6, sense oligonucleotide, progesterone treated; lanes 3 and 7, antisense oligonucleotide, no progesterone; lanes 4 and 8, antisense oligonucleotide, progesterone treated. In both (A) and (B), each lane contains RNA isolated from a single oocyte. Similar results were obtained in two separate experiments, using different frogs.

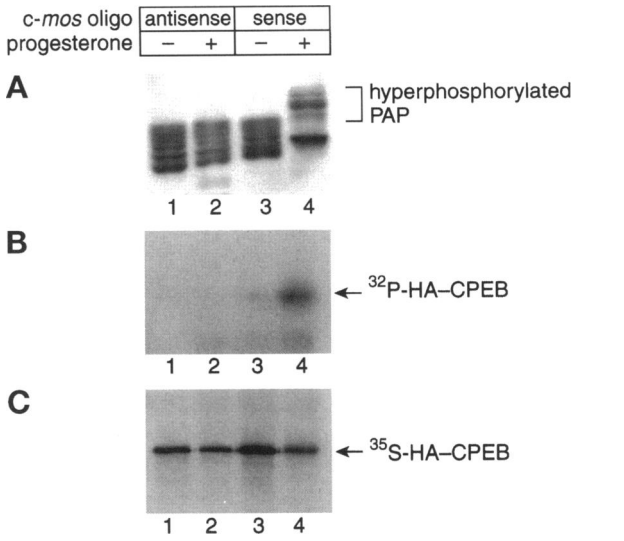

Figure 9. Hyper-phosphorylation of PAP or CPEB is not required for class I polyadenylation. Effect of blocking c-*mos* translation on progesterone-induced phosphorylation of endogenous PAP (A) or exogenous CPEB (B and C). Injection of antisense oligonucleotide complementary to c-*mos* mRNA was used to block class II polyadenylation, while still allowing class I polyadenylation, as in Figure 3. Sense-strand oligonucleotides were injected as controls. Lane 1, antisense oligonucleotide, no progesterone; lane 2, antisense oligonucleotide, progesterone treated; lane 3, sense oligonucleotide, no progesterone; lane 4, sense oligonucleotide, progesterone treated. (A) Immunoblot comparing mobilities of oocyte PAP proteins in response to progesterone treatment. A monoclonal anti-PAP antibody, NN2, was used to detect the change in PAP mobility that occurs after phosphorylation during oocyte maturation (Ballantyne *et al.*, 1995). Each lane contains one oocyte equivalent of total protein (~20 μg). (B and C) Immunoprecipitation experiment to monitor the phosphorylation (B) and expression (C) of exogenous, epitope-tagged CPEB (HA-CPEB) in response to progesterone treatment. Oocytes were given injections of synthetic HA-CPEB mRNA and incubated in [^{35}S]Met/Cys and [^{32}P]orthophosphate. Cells were incubated overnight in the presence (even lanes) or absence (odd lanes) of progesterone. Radiolabeled proteins (5 cell equivalents) were recovered with a monoclonal anti-HA antibody, 12CA5, separated by SDS-PAGE, and visualized by autoradiography. (B) ^{32}P was distinguished from total radiolabel (C) by its ability to penetrate through four sheets of X-ray film. (C) ^{32}P plus ^{35}S, determined by analyzing film placed directly against the dried gel.

positive-acting polyadenylation factors that catalyze them. Class II mRNAs might be polyadenylated by the same factors as class I mRNAs, but be hidden from that apparatus by sequence-specific repressors. In this view, c-*mos* and MPF would relieve that repression. Alternatively, class II mRNAs might exploit a different polyadenylation factor, or require a higher activity of the same apparatus that catalyzes class I reactions. The observation that subtle changes in a CPE significantly influence class I versus class II behavior is accommodated most simply by a difference in positive-acting factors.

By definition, events required to activate class I must occur in the absence of c-*mos* mRNA's polyade-

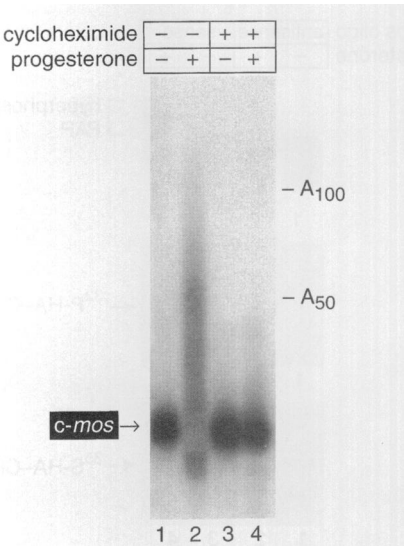

Figure 10. Protein synthesis is required for class I polyadenylation. Protein synthesis was inhibited by incubating oocytes for 2 h in 15 μg/ml cycloheximide; control oocytes were incubated in media lacking cycloheximide. Cycloheximide treatment reduced translation over 95% relative to control cells, as determined by incorporation of [^{35}S]methionine and [^{35}S]cysteine into trichloracetic acid (TCA) precipitates (see MATERIALS AND METHODS). Oocytes were given injections of 5 fmol of ^{32}P-labeled c-*mos* RNA. Cells were incubated overnight in the presence (even lanes) or absence (odd lanes) of progesterone. Lane 1, no cycloheximide, no progesterone; lane 2, no cycloheximide, progersterone treated; lane 3, cycloheximide treated, no progesterone; lane 4 cycloheximide treated, progesterone treated. Each lane contains RNA isolated from a single oocyte. Similar results were obtained in two separate experiments, using different frogs. Lengths of poly(A) added are indicated to the right of the panel. The position of the injected RNA is indicated to the left.

nylation and in the presence of the dominant negative form of CDK1. By these criteria, activation of class I polyadenylation is independent of detectable phosphorylation of CPEB and PAP. Cycloheximide treatment prevents polyadenylation of c-*mos* (Figure 10) and G10 (McGrew and Richter, 1990) RNAs in response to progesterone. Cytoplasmic polyadenylation of injected G10 RNA occurs early in meiotic maturation (McGrew and Richter, 1990), suggesting that it may be a class I event. The sensitivity of c-*mos* RNA's polyadenylation to cycloheximide implies that at least one mRNA must be translated to activate class I reactions. That mRNA might encode a protein that already is present in the oocyte but must be continually synthesized, or a new protein induced by progesterone treatment.

Sequences that are sufficient to specify which class of polyadenylation is used reside in the 3′-UTR, upstream of AAUAAA (Figure 7). Insertion of a simple CPE, UUUUUAU, confers class I behavior, and a single nucleotide deletion in a CPE can largely shift an RNA from class I to class II. These data strongly imply that CPEs themselves are sufficient to determine the activation mechanism used. However, sequences outside the CPE (or 3′-UTR) may also be critical, because D7 RNA, which contains UUUUUAU, exhibits class II behavior (Figure 6D).

Our results provide a technical approach that may help unravel the complex connections between poly(A) and translation using endogenous mRNAs. Although cytoplasmic polyadenylation is required for translational activation of certain mRNAs, it is dispensable for activation of others, at least in vitro (e.g., Standart *et al.*, 1990; for discussion, see Standart and Jackson, 1994; Wickens *et al.*, 1996). We have proposed that, for certain mRNAs, polyadenylation sustains or regulates translational activation that was achieved through independent means; for others, the role of polyadenylation may not be to stimulate translation, but instead to circumvent deadenylation, translational inactivation, and/or mRNA decay (Wickens *et al.*, 1996, 1997). By manipulating c-*mos* polyadenylation and CDK1 kinase, as described here, it should be possible to discern the contribution of poly(A) to translation of a variety of endogenous mRNAs in vivo. For example, using antisense c-*mos* oligonucleotides, Culp and Musci (unpublished data) observe that polyadenylation of FGF receptor mRNA (a class I mRNA) is insufficient to increase its translation in the absence of c-*mos* and MPF. Conversely, cyclin B1 protein levels increase normally in c-*mos* ablated oocytes treated

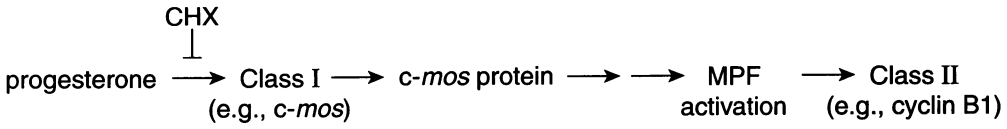

Figure 11. A dependent pathway comprising two classes of polyadenylation, and linking their activation to c-*mos* and MPF activity. The cytoplasmic polyadenylation of class I mRNAs, such as c-*mos* mRNA, occurs independently of, and before, accumulation of c-*mos* protein and activation of MPF. It is required for both events, through its stimulation of c-*mos* translation. Cycloheximide (CHX) blocks progesterone-induced polyadenylation of class I mRNA. Cytoplasmic polyadenylation of class II mRNAs, such as cyclin B1 mRNA, requires activation of c-*mos* and MPF. The connection between elevated levels of c-*mos* protein and activation of MPF likely involves multiple steps, as implied by the two arrows, but are not depicted in the figure (reviewed in Sagata, 1997). A possible feedback loop in which the initial elevation of c-*mos* levels is amplified is discussed in the text, but not depicted here. We do not intend to imply that the function of Class I and II polyadenylation reactions is invariably to stimulate translation (see Wickens *et al.*, 1996; Wickens *et al.*, 1997).

with progesterone, implying that polyadenylation is not required for its translational activation (unpublished results).

The simple cascade of reactions depicted in Figure 11 likely represents only some of the steps in a hierarchy of interdependent poly(A) addition and removal reactions. The nature of the mechanism that activates class I polyadenylation, and of the factors on which it acts, now is a central issue. These factors not only initiate the cascade of polyadenylation reactions during early development, but link those events to control of the meiotic and embryonic cell cycle.

ACKNOWLEDGMENTS

We thank Angel Nebreda and Tim Hunt for plasmids encoding wild-type and mutant forms of CDK1 K33R, and Kris Dickson for CPEB cDNA. We are grateful to Aaron Barkoff, Niki Gray, and Sunnie Thompson for reading the manuscript, and to all members of the Wickens laboratory for helpful discussions. We thank Aaron Barkoff for assistance with some of the experiments, and Angel Nebreda, Tim Hunt, Patricia Culp, and Tom Musci for communication of results before publication. We are grateful to Laura vander Ploeg, Robin Davies, and Adam Steinberg of the University of Wisconsin Biochemistry Media Lab for preparing figures. S.B. was supported in part by a predoctoral fellowship from an National Institutes of Health Training Grant in Molecular Biosciences. This work was supported by National Institutes of Health Grants RO1-GM31892 and RO1-GM50942 to M.W.

REFERENCES

Audic, Y., Omilli, R., and Osborne, H. (1997). Post-fertilization deadenylation of mRNAs in *Xenopus laevis* is sufficient to cause their degradation at the blastula stage. Mol. Cell. Biol. *17*, 209–218.

Ballantyne, S., Bilger, A., Åstrom, J., Virtanen, A., and Wickens, M. (1995). Poly(A) polymerases in the nucleus and cytoplasm of frog oocytes: dynamic changes during oocyte maturation and early development. RNA *1*, 64–78.

Bilger, A., Fox, C.A., Wahle, E., and Wickens, M. (1994). Nuclear polyadenylation factors recognize cytoplasmic polyadenylation elements. Genes Dev. *8*, 1106–1116.

Colgan, D., Murthy, K.G., Prives, C., and Manley, J. (1996). Cell-cycle related regulation of poly(A) polymerase by phosphorylation. Nature *384*, 282–285.

Curtis, D., Lehmann, R., and Zamore, P.D. (1995). Translational regulation in development. Cell *81*, 171–178.

Daar, I., Paulse, R.S., and Vande Woude, G.F. (1991). A characterization of cytostatic factor activity from *Xenopus* eggs and c-mos transformed cells. J. Cell Biol. *114*, 329–335.

Dworkin, M.B., and Dworkin, R.E. (1985). Changes in RNA titers and polyadenylation during oogenesis and oocyte maturation in *Xenopus laevis*. Dev. Biol. *112*, 451–457.

Dworkin, M.B., Shrutkowski, A., and Dworkin, R.E. (1985). Mobilization of specific maternal RNA species into polysomes after fertilization in *Xenopus laevis*. Proc. Natl. Acad. Sci. USA *82*, 7636–7640.

Fox, C., and Wickens, M. (1990). Poly(A) removal during oocyte maturation: a default reaction selectively prevented by specific sequences in the 3′ UTR of certain maternal mRNAs. Genes Dev. *4*, 2287–2298.

Fox, C.A., Sheets, M.D., Wahle, E., and Wickens, M. (1992). Polyadenylation of maternal mRNA during oocyte maturation: poly(A) addition in vitro requires a regulated RNA binding activity and a poly(A) polymerase. EMBO J. *11*, 5021–5032.

Fox, C.A., Sheets, M.D., and Wickens, M. (1989). Poly(A) addition during maturation of frog oocytes: distinct nuclear and cytoplasmic activities and regulation by the sequence UUUUUAU. Genes Dev. *3*, 2151–2162.

Furuno, N., Nishizawa, M., Okazaki, K., Tanaka, H., Iwashita, J., Nakajo, N., Ogawa, Y., and Sagata, N. (1994). Suppression of DNA replication via Mos function during meiotic divisions in *Xenopus* oocytes. EMBO J. *13*, 2399–2410.

Gebauer, F., and Richter, J.D. (1995). Cloning and characterization of a *Xenopus* poly(A) polymerase. Mol. Cell. Biol. *15*, 1422–1430.

Gebauer, F., Xu, W., Cooper, G., and Richter, J.D. (1994). Translational control by cytoplasmic polyadenylation of *c-mos* mRNA is necessary for oocyte maturation in the mouse. EMBO J. *13*, 5712–5720.

Gotoh, Y., Masuyama, N., Dell, K., Shirakabe, K., and Nishida, E. (1995). Initiation of *Xenopus* oocyte maturation by activation of the mitogen-activated protein kinase cascade. J. Biol. Chem. *270*, 25898–25904.

Hake, L., and Richter, J. (1994). CPEB is a specificity factor that mediates cytoplasmic polyadenylation during *Xenopus* oocyte maturation. Cell *79*, 617–627.

Huarte, J., Stutz, A., O'Connell, M.L., Gubler, P., Belin, D., Darrow, A.L., Strickland, S., and Vassalli, J-D. (1992). Transient translational silencing by reversible mRNA deadenylation. Cell, *69*, 1021–1030.

Hyman, L.E., and Wormington, W.M. (1988). Translational inactivation of ribosomal protein mRNAs during *Xenopus* oocyte maturation. Genes Dev. *2*, 598–605.

Kanki, J.P., and Donoghue, D.J. (1991). Progression from meiosis I to meiosis II in *Xenopus* oocytes requires de novo translation of the mos protooncogene. Proc. Natl. Acad. Sci. USA *88*, 5794–5798.

Kobayashi, H., Minshull, J., Ford, C., Golsteyn, R., Poon, R., and Hunt, T. (1991). On the synthesis and destruction of A- and B-type cyclins during oogenesis and meiotic maturation in *Xenopus laevis*. J. Cell Biol. *114*, 755–765.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. Nature *227*, 680–685.

Macdonald, P., and Smibert, C.A. (1996). Translational regulation of maternal mRNAs. Curr. Opin. Gen. Dev. *6*, 403–407.

McGrew, L.L., Dworkin, R.E., Dworkin, M.B., and Richter, J.D. (1989). Poly(A) elongation during *Xenopus* oocyte maturation is required for translational recruitment and is mediated by a short sequence element. Genes Dev. *3*, 803–815.

McGrew, L.L., and Richter, J.D. (1990). Translational control by cytoplasmic polyadenylation during *Xenopus* oocyte maturation: characterization of cis and trans elements and regulation by cyclin/MPF. EMBO J. *9*, 3743–3751.

Melton, D., Krieg, P., Rebagliati, M., Maniatis, T., Sinn, K., and Green, M. (1984). Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Res. *12*, 7035–7056.

Murray, A.W., and Kirschner, M. (1989). Cyclin synthesis drives the early embryonic cell cycle. Nature *339*, 275–280.

Murray, A.W., and Hunt, T. (1993). The Cell Cycle: An Introduction, New York: W.H. Freeman and Co.

Nebreda, A.R., Gannon, J.V., and Hunt, T. (1995). Newly synthesized protein(s) must associate with p34cdc2 to activate MAP kinase and MPF during progesterone-induced maturation of *Xenopus* oocytes. EMBO J. *14*, 5597–5607.

Nigg, E.A. (1995). Cyclin-dependent protein kinases: key regulators of the eukaryotic cell cycle. Bioessays *17*, 471–480.

Paris, J., and Philippe, M. (1990). Poly(A) metabolism and polysomal recruitment of maternal mRNAs during early *Xenopus* development. Dev. Biol. *140*, 221–224.

Paris, J., and Richter, J.D. (1990). Maturation-specific polyadenylation and translational control: diversity of cytoplasmic polyadenylation elements, influence of poly(A) tail size, and formation of stable polyadenylation complexes. Mol. Cell. Biol. *10*, 5634–5645.

Paris, J., Swenson, K., Piwnica-Worms, H., and Richter, J.D. (1991). Maturation-specific polyadenylation: in vitro activation by p34cdc2 and phosphorylation of a 58-kD CPE-binding protein. Genes Dev. *5*, 1697–1708.

Pickham, K.M., Meyer, A., Li, J., and Donoghue, D.J. (1992). Requirement of mos protein kinase for meiotic maturation of *Xenopus* oocytes induced by a *cdc2* mutant lacking regulatory phosphorylation sites. Mol. Cell. Biol. *12*, 3192–3203.

Pines, J., and Hunt, T. (1987). Molecular cloning and characterization of the mRNA for cyclin from sea urchin eggs. EMBO J. *6*, 2987–2995.

Pines, J., and Hunter, T. (1990). Human cyclin A is adenovirus E1A-associated protein p60 and behaves differently from cyclin B. Nature *346*, 760–763.

Richter, J.D. (1996). Dynamics of poly(A) addition and removal during development, Translational Control, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Sagata, N. (1997). What does Mos do in oocytes and somatic cells? Bioessays *19*, 13–21.

Sagata, N., Oskarsson, M., Copeland, T., Brumbaugh, J., and Vande Woude, W.G.F. (1988). Function of c-mos proto-oncogene product in meiotic maturation in *Xenopus* oocytes. Nature *335*, 519–525.

Sagata, N., Watanabe, N., Vande Woude, G., and Ikawa, Y. (1989). The c-*mos* proto-oncogene product is a cytostatic factor responsible for meiotic arrest in vertebrate eggs. Nature *342*, 512–518.

Salles, F.J., Darrow, A.L., O'Connell, M.L., and Strickland, S. (1992). Isolation of novel murine maternal mRNAs regulated by cytoplasmic polyadenylation. Genes Dev. *6*, 1202–1212.

Sanger, F., Nicklen, S., and Coulson, A.R. (1977). DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA *74*, 5463–5468.

Sheets, M., Wu, M., and Wickens, M. (1995). Polyadenylation of c-*mos* mRNA as a control point in *Xenopus* meiotic maturation. Nature *374*, 511–516.

Sheets, M.D., Fox, C.A., Hunt, T., Vande Woude, G., and Wickens, M. (1994). The 3′-untranslated regions of c-mos and cyclin mRNAs stimulate translation by regulating cytoplasmic polyadenylation. Genes Dev. *8*, 926–938.

Smith, R.C., Dworkin, M.B., and Dworkin, R.E. (1988). Destruction of a translationally controlled mRNA in *Xenopus* oocytes delays progesterone-induced maturation. Genes Dev. *2*, 1296–1306.

Smith, R.C., Dworkin, R.E., and Dworkin, M.B. (1988). Expression of a histone H1-like protein is restricted to early *Xenopus* development. Genes Dev. *2*, 1284–1295.

Solomon, M.J., Lee, T., and Kirschner, M.W. (1992). Role of phosphorylation in p34cdc2 activation: identification of an activating kinase. Mol. Biol. Cell *3*, 13–27.

Standart, N., Dale, M., Stewart, E., and Hunt, T. (1990). Maternal mRNA from clam oocytes can be specifically unmasked in vitro by antisense RNA complementary to the 3′ untranslated region. Genes Dev. *4*, 2157–2168.

Standart, N., and Jackson, R. (1994). Regulation of translation by specific protein/mRNA interactions. Biochimie *76*, 867–879.

Stebbins-Boaz, B., Hake, L.E., and Richter, J.D. (1996). CPEB controls the cytoplasmic polyadenylation of cyclin, Cdk2 and c-*mos* mRNAs and is necessary for oocyte maturation in *Xenopus*. EMBO J. *15*, 2582–2592.

Stebbins-Boaz, B., and Richter, J.D. (1994). Multiple sequence elements and a maternal mRNA product control cdk2 RNA polyadenylation and translation during early *Xenopus* development. Mol. Cell. Biol. *14*, 5870–5880.

Sturgess, E.A., Ballantine, J.E., Woodland, H.R., Mohun, P.R., Lane, C.D., and Dimitriadis, G.J. (1980). Actin synthesis during the early development of *Xenopus laevis*. J. Embryol. Exp. Morphol. *58*, 303–320.

Swenson, K.I., Farrell, K.M., and Ruderman, J.V. (1986). The clam embryo protein cyclin A induces entry into M phase and the resumption of meiosis in *Xenopus* oocytes. Cell *47*, 861–870.

Thuresson, A.-C., Åström, J., Åström, A., and Gronvik, K.-O. (1994). Multiple forms of poly(A) polymerases in human cells. Proc. Natl. Acad. Sci. USA *91*, 979–983.

Varnum, S.M., Hurney, C.A., and Wormington, M.W. (1992). Maturation-specific deadenylation in *Xenopus* oocytes requires nuclear and cytoplasmic factors. Dev. Biol. *153*, 283–290.

Varnum, S.M., and Wormington, M.W. (1990). Deadenylation of maternal mRNAs during *Xenopus* oocyte maturation does not require specific cis-sequences: a default mechanism for translational control. Genes Dev. *4*, 2278–2286.

Wasserman, W.J., and Masui, Y. (1975). Effects of cycloheximide on a cytoplasmic factor initiating meiotic maturation in *Xenopus* oocytes. Exp. Cell Res. *91*, 381–388.

Weeks, D.L., Walder, J.A., and Dagle, J.M. (1991). Cyclin B mRNA depletion only transiently inhibits the *Xenopus* embryonic cell cycle. Development *111*, 1173–1178.

Westendorf, J.M., Swenson, K.I., and Ruderman, J.V. (1989). The role of cyclin B in meiosis I. J. Cell Biol. *108*, 1431–1444.

Whitfield, W.G., Gonzalez, C., Maldonado-Codina, G., and Glover, D.M. (1990). The A and B type cyclins of *Drosophila* are accumulated and destroyed in temporally distinct events that define separable phases of the G2-M transition. EMBO J. *9*, 2563–2572.

Wickens, M. (1992). Forward, backward, how much, when: mechanisms of poly(A) addition and removal and their role in early development. Semin. Dev. Biol. *3*, 399–412.

Wickens, M., Kimble, J., and Strickland, S. (1996). Translational control of developmental decisions, Translational Control, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Wickens, M., Anderson, P., and Jackson, R. (1997). Life and death in the cytoplasm: messages from the 3′ end. Curr. Opin. Genet. Dev. *7*, 220–232.

Wormington, W.M. (1989). Developmental expression and 5S rRNA-binding activity of *Xenopus laevis* ribosomal protein L5. Mol. Cell. Biol. *9*, 5281–5288.

Yew, N., Mellini, M.L., and Vande Woude, G.F.W. (1992). Meiotic initiation by the *mos* protein in *Xenopus*. Nature *355*, 649–652.