

Maturation-Specific Polyadenylation and Translational Control: Diversity of Cytoplasmic Polyadenylation Elements, Influence of Poly(A) Tail Size, and Formation of Stable Polyadenylation Complexes

JEANNIE PARIS AND JOEL D. RICHTER*

Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545

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Early embryonic development in *Xenopus laevis* is programmed in part by maternally derived mRNAs, many of which are translated at the completion of meiosis (oocyte maturation). Polysomal recruitment of at least one of these mRNAs, G10, is regulated by cytoplasmic poly(A) elongation which, in turn, is dependent upon the cytoplasmic polyadenylation element (CPE) UUUUUUAUAAAAG and the hexanucleotide AAUAAA (L. L. McGrew, E. Dworkin-Rastl, M. B. Dworkin, and J. D. Richter, *Genes Dev.* 3:803–815, 1989). We have investigated whether sequences similar to the G10 RNA CPE that are present in other RNAs could also be responsible for maturation-specific polyadenylation. B4 RNA, which encodes a histone H1-like protein, requires a CPE of the sequence UUUUUAAU as well as the polyadenylation hexanucleotide. The 3' untranslated regions of *Xenopus c-mos* RNA and mouse HPRT RNA also contain U-rich CPEs since they confer maturation-specific polyadenylation when fused to *Xenopus* B-globin RNA. Polyadenylation of B4 RNA, which occurs very early during maturation, is limited to 150 residues, and it is this number that is required for polysomal recruitment. To investigate the possible diversity of factors and/or affinities that might control polyadenylation, egg extracts that faithfully adenylate exogenously added RNA were used in competition experiments. At least one factor is shared by B4 and G10 RNAs, although it has a much greater affinity for B4 RNA. Additional experiments demonstrate that an intact CPE and hexanucleotide are both required to compete for the polyadenylation apparatus. Gel mobility shift assays show that two polyadenylation complexes are formed on B4 RNA. Optimal complex formation requires an intact CPE and hexanucleotide but not ongoing adenylation. These data, plus additional RNA competition studies, suggest that stable complex formation is enhanced by an interaction of the *trans*-acting factors that bind the CPE and polyadenylation hexanucleotide.

The biogenesis of translationally competent mRNAs usually requires extensive posttranscriptional processing (18). One processing event in particular, polyadenylation, takes place in cell nuclei on almost all pre-mRNAs. In the past few years, however, it has become clear that polyadenylation can also occur on some cytoplasmic mRNAs primarily during embryonic development. This was suggested initially by observations that maternal mRNAs in sea urchin eggs (27) and *Xenopus* oocytes (1, 4) undergo large changes in poly(A) tail size in response to fertilization and oocyte maturation, respectively. More recently, Northern (RNA) blot analysis with transcript-specific probes has shown that several translationally dormant (masked) mRNAs in eggs and oocytes become polyadenylated and enter polysomes in response to fertilization or oocyte maturation (5, 8, 14, 19, 21, 22). Conversely, other polyadenylated mRNAs that are polysomal in eggs or oocytes are deadenylated during fertilization or maturation and are no longer translated (9, 19, 21, 22). It therefore appears that poly(A) metabolism during early development is instrumental in controlling the translation of specific mRNAs.

Detailed analysis of maturation-specific polyadenylation and its effects on translation has been described for two mRNAs. The first, which encodes tissue plasminogen activator, is polyadenylated and recruited for translation during

mouse oocyte maturation (8). Oocyte injection studies have demonstrated that the activation of this mRNA is regulated by the number of 3' adenylate residues; mRNAs that already contain a long poly(A) tail are translated in oocytes that do not undergo maturation. The signals that normally confer polyadenylation reside in the 3' untranslated region and include the hexanucleotide AAUAAA, which has been shown previously to be important for positioning the site of cleavage and subsequent polyadenylation for nuclear pre-mRNAs (26). It is unlikely, however, that this is the only signal that confers maturation-specific polyadenylation, since AAUAAA is present in virtually all mRNAs regardless of whether they undergo this developmentally regulated processing (13).

A second mRNA, termed G10, is polyadenylated and translated during *Xenopus* oocyte maturation (14). In contrast to tissue plasminogen activator mRNA, polysomal recruitment of G10 mRNA is regulated by the active process of polyadenylation rather than by the length of the poly(A) tail (14). Polyadenylation of this mRNA is controlled by two elements in the 3' untranslated region, the polyadenylation hexanucleotide AAUAAA and a U-rich sequence termed the cytoplasmic polyadenylation element (CPE) (14; L. L. McGrew and J. D. Richter, *EMBO J.*, in press). The CPE as defined for G10 mRNA is UUUUUUAUAAAAG, whereas that for another RNA that is polyadenylated during *Xenopus* maturation, D7 RNA, is UUUUUUAU (6). The observations that these CPEs are only partially overlapping and that the

* Corresponding author.

mRNAs in which they reside are polyadenylated at different times during maturation (6; McGrew and Richter, in press) might suggest a diversity of interacting factors and/or affinities.

In this study, we have used injected *Xenopus* oocytes to show that the 3' untranslated regions of mRNAs encoding a histonelike protein called B4 (25), the proto-oncogene *c-mos* (23), and mouse HPRT (10, 19) contain a U-rich CPE plus the hexanucleotide AAUAAA, which are required for polyadenylation during oocyte maturation. RNAs containing these CPEs are polyadenylated in a hierarchical manner with respect to both the number of molecules adenylated and the number of residues polymerized per molecule. Analysis of B4 RNA translation shows that it is regulated by the length of its poly(A) tail and not by ongoing adenylation, which is the case for G10 RNA (12). In addition, B4 RNA polyadenylation occurs very early in the maturation process and does not require nuclear components. To assess the possible diversity of factors and/or affinities that regulate polyadenylation, we have used egg extracts that faithfully adenylate exogenously added RNA. Results of competition studies in vitro suggest that cellular factors can distinguish between different CPEs but that at least one component of the polyadenylation machinery that is rate limiting is shared by G10 and B4 RNAs. Finally, analysis of polyadenylation complexes by gel mobility shift assays indicates a stable interaction between *trans*-acting factors that bind the CPE and hexanucleotide AAUAAA. The implications of these findings for polyadenylation and translational control during oocyte maturation are discussed.

MATERIALS AND METHODS

RNA extraction and Northern blot analysis. Total RNA was extracted from *Xenopus laevis* stage VI oocytes (3) and eggs as described by McGrew et al. (14). To deadenylate RNA in vitro, 20 µg of total RNA was incubated with 1 µg of oligo(dT)₁₂₋₁₈ and digested with RNase H by the procedure of Colot and Rosbash (1). The RNAs were then extracted with phenol and chloroform, resolved by electrophoresis through agarose-formaldehyde gels by the method of Meinholt and Wahl (15), and blotted onto nitrocellulose (12). Plasmid pB4-2, which was cloned into the *Xho*I site of pBluescript SK⁻ (generously provided by Rosamund Smith), was linearized with *Bgl*III and used to synthesize a [³²P]UTP-labeled transcript with T₃RNA polymerase (Stratagene). The Northern blot was hybridized with this probe by the procedure of Melton et al. (16).

Plasmid constructions and RNA synthesis. A *Xenopus* B-globin cDNA used to generate chimeric RNAs was derived from plasmid pSP64XBM (16). This plasmid was digested with *Bst*EII and *Xba*I, and the large fragment, which contains vector sequences plus all globin sequences except the 3' untranslated region and 6 bases upstream of the stop codon, was isolated. These restriction enzyme sites were used for the ligation of annealed synthetic oligonucleotides encoding selected regions of the 3' untranslated regions of *Xenopus* B4-2 RNA, nucleotides 1128 to 1158 (25); *Xenopus c-mos* RNA, nucleotides 3000 to 3021 (23); and mouse HPRT RNA, nucleotides 1166 to 1189 (10). For the sequences of the inserted oligonucleotides, see Fig. 2A. In some cases, mutations were inserted in the 3' noncoding region of B4 RNA (see Fig. 3A). The procedures for the cloning of synthetic oligonucleotides have been described previously (14). The construction of pXBG10-360 has been described by McGrew and Richter (in press). The transcripts

generated from these chimeric plasmids by SP6 polymerase are 524 to 535 bases long.

To study the time course of polyadenylation in vivo, cDNAs encoding a wild-type small Gb/B4 RNA and small Gb/B4 RNAs with mutations in the CPE and polyadenylation hexanucleotide were constructed. The plasmids pGb/B4-WT, pGb/B4-M2, and pGb/B4-M4 were digested with *Nco*I and *Bst*EII; the large fragment, which lacks globin-coding sequences, was isolated, filled in with the Klenow fragment of DNA polymerase I, and ligated. The transcript generated from this plasmid by SP6 polymerase is 110 bases long.

DNA templates were linearized with *Eco*RI (pB4-2) or *Xba*I (all globin RNA chimeric derivatives). Transcription reactions with T7 or SP6 polymerases were carried out as described by Krieg and Melton (11), except that the mixture contained 250 µM GpppG, 50 µM UTP, 50 µM GTP, 250 µM CTP, 250 µM ATP, and 40 µCi of [³²P]UTP (800 Ci/mmol; Du Pont, NEN Research Products). Following synthesis, the RNA was extracted with phenol-chloroform, precipitated with ethanol, and suspended in water at a concentration of about 0.1 mg/ml.

Gb/B4 RNA was also modified in vitro to contain a poly(A) tail. A 50-ng portion of this labeled transcript was incubated in a total volume of 20 µl with 0.2 mM ATP, 2 mM MgCl₂, 5 mM dithiothreitol, 40 mM NaCl, 100 mM Tris hydrochloride (pH 8), 1 U of RNasin per µl, and 1 U of poly(A) polymerase (Pharmacia) for 1 to 3 min at 37°C. The reaction was stopped by the addition of 400 µl of 0.2 M sodium acetate, 0.1% sodium dodecyl sulfate (SDS), and 2 µg of yeast RNA. The RNA was extracted with phenol and chloroform and precipitated with ethanol.

Oocyte injection, polysome preparation, and RNA extraction. Manually defolliculated stage VI oocytes were injected with about 40 nl of solution containing radiolabeled RNA (10⁴ dpm/ng) and cultured in Barth medium. Some oocytes were also cultured in medium containing progesterone (1 µg/ml) to induce maturation, which usually occurred in 5 to 8 h and was scored by the presence of a white spot at the animal pole. Total RNA was then extracted with phenol and chloroform and analyzed without further treatment. Alternatively, the RNA was hybridized with oligo(dT) and treated with RNase H to remove the poly(A) tail (1). Some injected oocytes were also homogenized and centrifuged through 10 and 35% sucrose (14). The RNA that was subsequently extracted from the pellet is considered to be polysomal, whereas that from the supernatant is considered to be nontranslating. Other oocyte homogenates were centrifuged through a 15 to 40% sucrose gradient in an SW41 tube for 2 h at 35,000 rpm. Fractions (1 ml) were collected, and the RNA was extracted. RNA was resolved by electrophoresis through 4% acrylamide containing 7 M urea and then by autoradiography.

Some oocytes were injected with about 10 ng of radioinert Gb/B4 RNA and incubated in the presence of progesterone until mature. They were then injected with [³⁵S]methionine (0.3 pmol; specific activity, 1,000 Ci/mmol) and incubated for another 1 h. The oocytes were homogenized, and the radiolabeled protein was resolved by SDS-15% polyacrylamide gel electrophoresis and fluorography.

Preparation of extracts. Oocyte and egg extracts were prepared as described originally by Murray and Kirschner (17). Briefly, dejellied *Xenopus* eggs were collected in buffer containing 100 mM KCl, 1 mM MgCl₂, 0.1 mM CaCl₂, 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; pH 7.7), 50 mM sucrose, 10 µg each of leupeptin,

chymostatin, and pepstatin per ml, and 100 μ g of cytochalasin B per ml. They were then centrifuged in an HB-4 rotor through Versilube F-50 oil (General Electric) to separate them from the buffer and then crushed by centrifugation at 10,000 rpm for 10 min in an HB-4 rotor. The cytosol layer was collected and then adjusted to 1 mM ATP, 1 mM $MgCl_2$, 7.5 mM creatine phosphate, 0.1 mM ethylene glycol-bis(β -aminoethyl ether)- N,N,N',N' -tetraacetic acid (EGTA; pH 7.7), 10 μ g of protease inhibitors per ml, and 10 μ g of cytochalasin B per ml. Following a final clarifying centrifugation (10,000 rpm for 15 min), the extract was frozen in aliquots at $-80^\circ C$. Approximately 0.1 ml of extract was obtained from 400 eggs.

Polyadenylation assays were typically performed in a 10- μ l reaction volume containing 3.5 μ l of extract, ca. 2 ng RNA (2×10^4 dpm), 100 mM KCl, 1 mM ATP, 2 mM $MgCl_2$, 0.1 mM $CaCl_2$, 7.5 mM creatine phosphate, 0.1 mM EGTA, and 10 mM HEPES (pH 7.7). The mixtures were incubated at $23^\circ C$ for 1 h. The reaction was stopped by the addition of 0.2 M sodium acetate and 0.1% SDS, and the RNA was extracted and analyzed as described previously.

Analysis of polyadenylation complexes. Prior to use, substrate RNAs were purified from a polyacrylamide gel slice by elution into 0.1% SDS-1 M ammonium acetate-10 mM Tris hydrochloride (pH 8) at room temperature for 5 to 12 h, phenol extracted, and ethanol precipitated. The RNAs were added to egg extracts as described above, except that at the end of the incubation, heparin was added to a final concentration of 5 mg/ml and the mixture was placed on ice for 10 min. Glycerol was then added to a final concentration of 8%, and the samples were immediately loaded onto a 3.5% polyacrylamide gel (acrylamide-to-bisacrylamide ratio, 80:1). The gel, which contained 50 mM Tris and 50 mM glycine, was preelectrophoresed in the same buffer for 30 min at 10 V/cm. Electrophoresis was carried out at the same voltage for 5 to 6 h, and the gel was then dried and autoradiographed.

RESULTS

Identification of sequence elements that confer maturation-specific polyadenylation. One characteristic of early *Xenopus* development is the coincident polyadenylation and mobilization of specific maternal mRNAs into polysomes (5, 14). An example of one such mRNA is B4 mRNA, which encodes a protein with homology to histone H1 (25). Northern blot analysis of total RNA from stage VI oocytes and eggs (mature oocytes) reveals that this RNA has increased in size by about 100 bases during maturation [Fig. 1A, Oligo(dT)]. To demonstrate that this size change was due at least partially to polyadenylation, RNA samples were annealed with oligo(dT) and digested with RNase H prior to Northern analysis. Following this treatment, B4 RNA from both oocytes and eggs had an identical electrophoretic migration rate. From a comparison with the migration rate of known size markers, we infer that oocyte B4 RNA has a poly(A) tail of about 50 residues that is increased to 150 residues during maturation.

To investigate the regulation of B4 RNA polyadenylation, we used a B4 cDNA clone that includes the entire 3' untranslated region inserted into a vector containing the T7 promoter. The cDNA was linearized downstream of the poly(A) tail and used to generate [^{32}P]RNA. To remove vector-derived sequences downstream of the poly(A) that could interfere with the polyadenylation reaction (14), we treated the transcript with oligo(dT) and RNase H, which should truncate the RNA immediately after the 3' untrans-

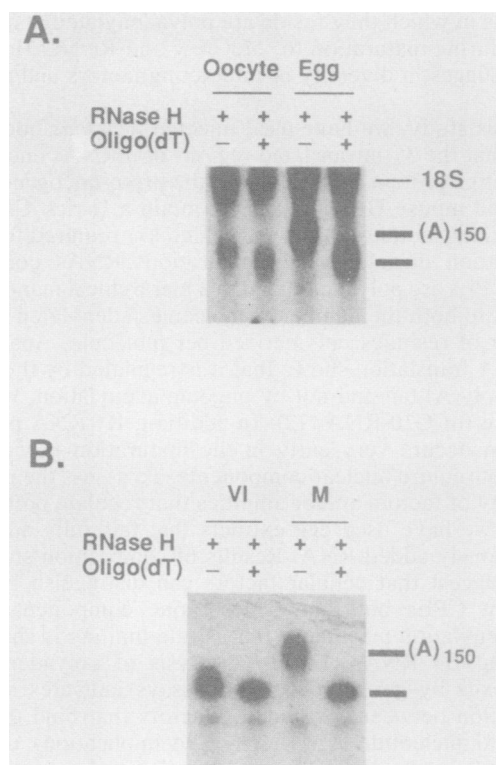


FIG. 1. Polyadenylation analysis of B4 RNA during oocyte maturation. (A) Total RNA was extracted from stage VI oocytes and shed eggs and treated with RNase H in the absence or presence of oligo(dT). The RNA was resolved by agarose-formaldehyde gel electrophoresis, blotted to nitrocellulose, and probed with an antisense ^{32}P -labeled B4 RNA. The 18S rRNA is denoted, as are the sizes of nonadenylated (thick line, ca. 1,200 bases) and adenylated [solid line plus (A)₁₅₀] B4 RNAs. (B) Oocytes were injected with ^{32}P -labeled B4 RNA and incubated in the absence (VI) or presence (M) of progesterone. Following maturation, RNA was extracted and treated with RNase H in the absence or presence of oligo(dT). The RNA was then resolved by electrophoresis through a 4% acrylamide-7 M urea gel and autoradiographed. Nonadenylated and adenylated B4 RNAs are denoted as in panel A.

lated region. When this transcript was injected into stage VI oocytes that were then induced to mature with progesterone, the RNA increased in size by about 150 bases (Fig. 1B). Since this size increase was sensitive to subsequent treatment with oligo(dT) and RNase H, we conclude that injected B4 RNA is regulated by the same polyadenylation apparatus as its endogenous counterpart.

An inspection of the 3' untranslated regions of several RNAs that we suspected would be polyadenylated during maturation led to the identification of a conserved U-rich sequence. Subsequent analysis showed this to be a control element for the polyadenylation of G10 RNA (14). We further demonstrated that this element, together with the polyadenylation hexanucleotide AAUAAA, was sufficient to confer polyadenylation to globin mRNA following oocyte injection and progesterone-induced maturation (14). To determine whether the U-rich sequence of other RNAs also controls maturation-specific polyadenylation, we synthesized chimeric mRNAs composed of globin-coding sequences followed by a portion of the 3' untranslated region of B4 RNA, mouse HPRT RNA, and *c-mos* RNA (Fig. 2A).

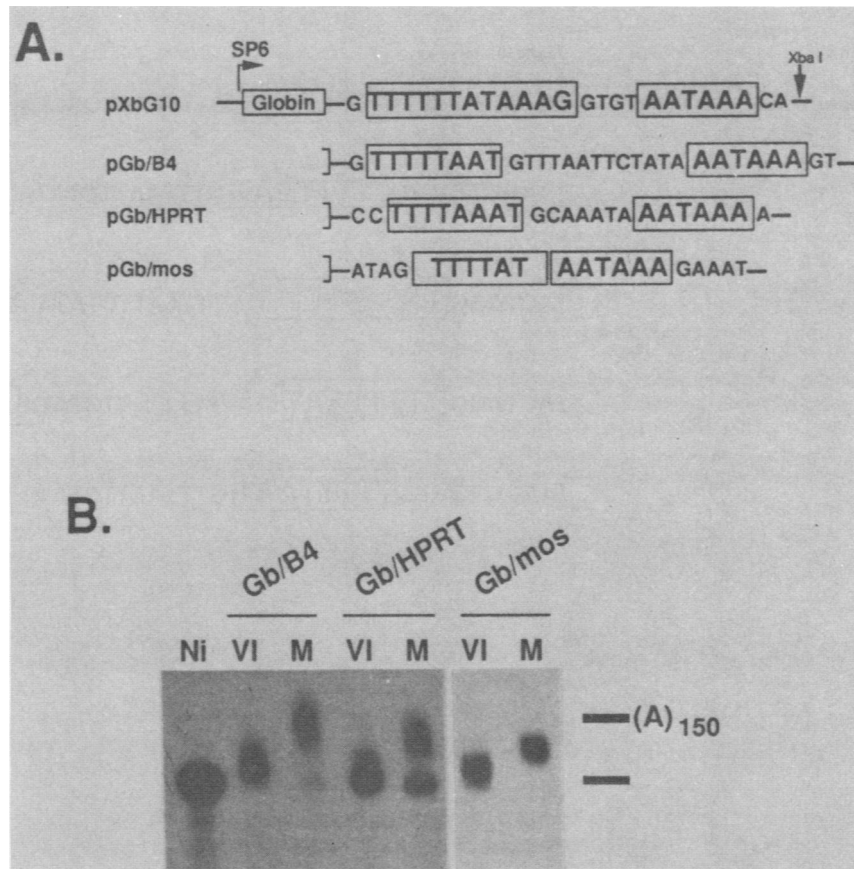


FIG. 2. Polyadenylation analysis of chimeric RNAs during oocyte maturation. (A) Partial sequence of chimeric DNAs composed of the corresponding 5' and coding regions of *Xenopus* B-globin RNA fused to a portion of DNA encoding the 3' untranslated region of B4, mouse HPRT, and *Xenopus c-mos* RNA. The boxes denote the known or predicted cytoplasmic polyadenylation elements and the polyadenylation hexanucleotide. For each construction, the DNA template was linearized at an *Xba*I site and the RNA was synthesized with Sp6 polymerase. (B) Radiolabeled chimeric RNAs that were synthesized from the DNAs described in panel A were injected into stage VI oocytes, some of which were induced to mature (M) with progesterone. RNA was then extracted and analyzed as in Fig. 1B. Ni refers to noninjected RNA, and the black lines refer to nonadenylated RNA (ca. 500 bases) and adenylated RNA as described in the legend to Fig. 1A.

In addition to containing a putative U-rich polyadenylation control element, B4 RNA was chosen because it undergoes a size increase during early development (4), *c-mos* RNA was chosen because it is translated specifically during oocyte maturation (23), and HPRT RNA was chosen because it is polyadenylated during mouse oocyte maturation (19). When injected into *Xenopus* oocytes, each of these chimeric RNAs was polyadenylated following maturation (Fig. 2B). Interestingly, the number of molecules adenylated and the number of residues polymerized varied for each RNA. Thus, only about 50% of the Gb/HPRT molecules were adenylated with 100 residues, whereas virtually all Gb/*mos* and Gb/B4 RNAs were adenylated with 50 and 150 residues, respectively. We conclude from these data that the 3' untranslated regions of these RNAs contain necessary polyadenylation control sequences; such sequences are tentatively identified as the U-rich CPE and the hexanucleotide AAUAAA (denoted in Fig. 2A).

To define more precisely the nucleotides that are required for B4 RNA adenylation, several transcripts were synthesized that carried various nucleotide substitution mutations (Fig. 3A). These RNAs were then injected into oocytes to determine whether they were polyadenylated during maturation (Fig. 3B). Although Gb/B4 RNA with the wild-type 3' untranslated region was efficiently polyadenylated, an RNA

harboring a U-to-G transition in the polyadenylation hexanucleotide was poorly adenylated (Gb/B4-M1 [Fig. 3B]). We inferred that a wild-type AAUAAA sequence is required for polyadenylation, but that a putative second hexanucleotide with the sequence UAUAAA (Fig. 3A) could compensate when the normal signal was mutated. This was shown to be the case because U-to-G conversions in both putative polyadenylation hexanucleotides (Gb/B4-M4 [Fig. 3A]) rendered the RNA refractory to polyadenylation (Gb/B4-M4 [Fig. 3B]). A substitution of the sequence UUUUAAU with a corresponding *Hind*III recognition site completely inhibited polyadenylation (Gb/B4-M2 [Fig. 3B]), whereas substitution of the adjacent 3' nucleotides had no effect (Gb/B4-M3). Therefore, Gb/B4 RNA, like G10 RNA (14; McGrew and Richter, submitted) and D7 RNA (6), requires two sequences for maturation-specific polyadenylation, a U-rich CPE and the hexanucleotide AAUAAA. It is interesting that the sequence UUUAAU, which resides between the CPE and polyadenylation hexanucleotide of B4 RNA, has no polyadenylation activity, since it did not substitute for the true CPE when it was converted to a *Hind*III site (Gb/B4-M2).

Nuclear pre-mRNA polyadenylation appears to be a two-step process; polymerization of the first 10 adenylate residues is AAUAAA dependent, whereas polymerization of the remaining residues is AAUAAA independent (24). To deter-

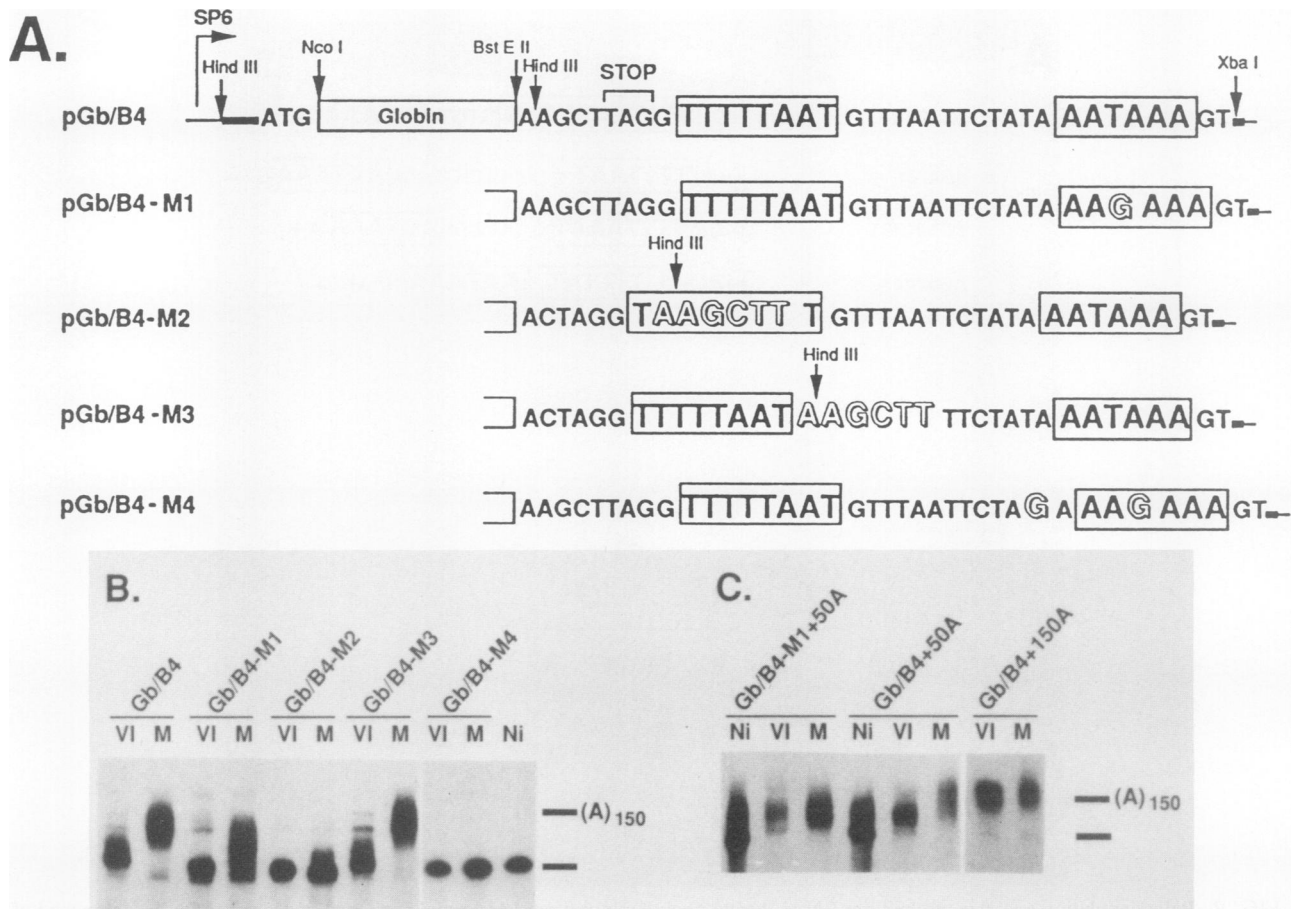


FIG. 3. Effect of mutations in Gb/B4 RNA on maturation-specific polyadenylation. (A) Sequence of DNAs corresponding to the 3' untranslated regions of chimeric globin/B4 RNAs. The symbols are as described in the legend to Fig. 2A, except that the sequence denoted by open letters refers to substituted nucleotides. (B) Radiolabeled wild-type or mutant B4 RNAs were injected into stage VI oocytes that were then matured with progesterone (M). Following maturation, the RNA was analyzed as described in the legend to Fig. 1A. (C) Wild-type or mutant Gb/B4 RNAs were adenylated in vitro with 50 or 150 residues. The RNAs were injected into oocytes, some of which were incubated in progesterone. Following maturation, the RNA was analyzed as described in the legend to Fig. 1A. Ni, Noninjected RNA.

mine whether B4 RNA polyadenylation is similarly regulated, we have added about 50 adenylate residues to two Gb/B4 RNAs in vitro with poly(A) polymerase; the first RNA contained wild-type *cis* signals, whereas the second contained a U-to-G transition in the hexanucleotide AAUAAA (Fig. 3C). Although the RNA with the normal hexanucleotide was further polyadenylated during maturation, an RNA containing the transition mutation in that sequence was not further adenylated. These data therefore indicate that pre-mRNA polyadenylation is distinct from that during oocyte maturation.

The data presented thus far show that endogenous or injected B4 RNAs (or derivatives) are adenylated up to 150 residues following oocyte maturation. One would therefore predict that the polyadenylation machinery can sense the number of adenylate residues on B4 RNA and would not adenylate this transcript if it already contained the mature number of residues. To test this hypothesis, we injected a Gb/B4 RNA that already contained 150 adenylate residues to see whether it would be further polyadenylated during oocyte maturation. Indeed, this RNA was not further adenylated and affirms that the polyadenylation machinery somehow regulates the length of the B4 RNA poly(A) tail to be 150 residues (Fig. 3C, lanes Gb/B4+150A).

Polyadenylation is an early event of maturation. *Xenopus*

oocyte maturation, which usually takes between 5 and 8 h, is scored by the appearance of a white spot at the animal pole that denotes germinal vesicle breakdown. Up to this point, we have analyzed polyadenylated RNA after the appearance of the white spot to ensure that only matured oocytes were examined. To determine the point during maturation at which Gb/B4 RNA is polyadenylated, we have analyzed RNA at several times following oocyte injection and incubation in medium containing progesterone. In this case, however, we have injected a Gb/B4 RNA composed of only 110 bases, which should resolve even small changes in adenylation. Polyadenylation was evident by 1 h postprogesterone and was progressive in terms of both adenylate residues added per RNA molecule and number of molecules adenylated (Fig. 4). Because germinal vesicle breakdown did not begin until 6 h, when nuclear components would be released into the cytoplasm (30% of the oocytes had a white spot at the animal pole at this time), one may infer that such material is not required for maturation-specific polyadenylation. A similar conclusion was reached by Fox et al. (6), who showed that manual enucleation did not inhibit polyadenylation.

Translational control by polyadenylation. Although there is a strong correlation between the polyadenylation of specific mRNAs during early *Xenopus* development and their re-

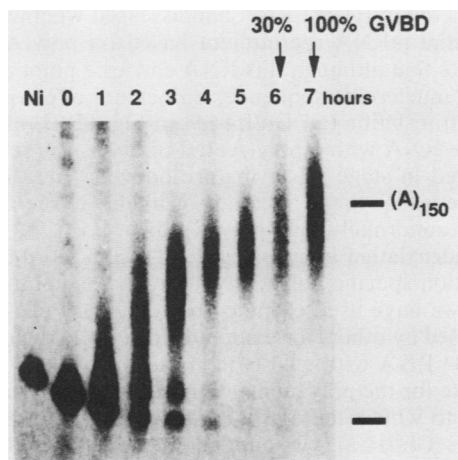


FIG. 4. Time course of polyadenylation during maturation. Labeled sGb/B4 RNA (a 110-base RNA derived from Gb/B4 RNA that lacks the entire globin-coding region) was injected into oocytes that were then incubated with progesterone. Every hour thereafter, RNA from three oocytes was extracted and analyzed as described in the text. The percentage of oocytes that had undergone germinal vesicle breakdown (GVBD), as assessed by a white spot at the animal pole, is indicated.

recruitment into polysomes (5, 14), the requirement of polyadenylation for translation has been demonstrated only for G10 RNA (14). We therefore have investigated the relationship between B4 RNA polyadenylation and translational recruitment during maturation. To first determine whether the 3' untranslated region of Gb/B4 RNA would in fact stimulate globin synthesis during maturation, we injected this mRNA into oocytes, some of which were then incubated in progesterone. Following the appearance of a white spot at the animal pole, the oocytes were injected with a pulse of [³⁵S]methionine. Globin synthesis was evident only in mature oocytes (Fig. 5A).

To assess the amount of Gb/B4 RNA on polysomes, oocytes injected with this radiolabeled mRNA were homogenized and centrifuged through a sucrose gradient (Fig. 5B). In stage VI (control) oocytes, less than 5% of the mRNA sedimented greater than the 80S monosome. Following maturation, not only was Gb/B4 RNA polyadenylated, as assessed by a slower electrophoretic migration compared with noninjected RNA (Fig. 5B, lane Ni), but about 50% of this material sedimented greater than 80S. Thus, recruitment of Gb/B4 RNA during maturation does indeed lead to the synthesis of globin.

To simplify the procedure for examining mRNA recruitment, we used centrifugation through a sucrose cushion rather than a gradient; the relative amounts of Gb/B4 RNA in the supernatant and pellet should also directly reflect the translational status of the mRNA during maturation. Although injected Gb/B4 was less than 10% polysomal in stage VI oocytes, it was nearly 50% polysomal in mature oocytes; this again demonstrates that after polyadenylation, the mRNA was much more efficiently recruited into polysomes (Fig. 6A). To ensure that RNA in the pellet was indeed associated with ribosomes, we treated some homogenates with EDTA (which dissociates polysomes) prior to centrifugation (Fig. 6A). Because this procedure eliminated Gb/B4 RNA in the pellet, we infer that under normal conditions, B4 RNA that sediments with polysomes is indeed actively translated.

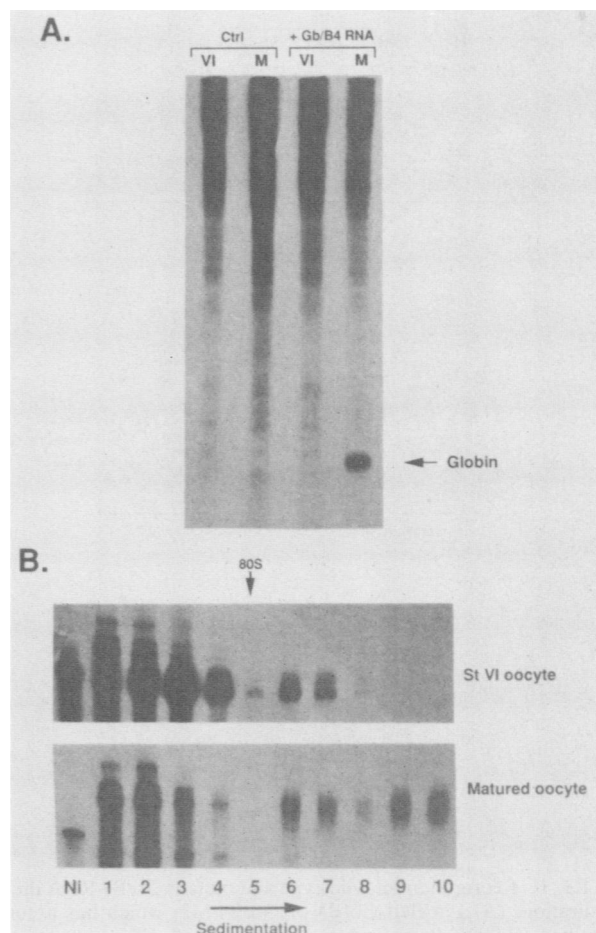


FIG. 5. Translation of Gb/B4 RNA during maturation. (A) Non-injected oocytes (Ctrl) and oocytes injected with Gb/B4 RNA were incubated with progesterone. Following maturation, the oocytes were injected with [³⁵S]methionine and incubated for 1 h. The oocytes were then homogenized, and the radiolabeled proteins were resolved by SDS-polyacrylamide gel electrophoresis and fluorography. The band representing globin is denoted. (B) Oocytes were injected with labeled Gb/B4 RNA, and one group was matured with progesterone. Control (St VI) and mature oocytes were then homogenized and centrifuged through a polysome-sucrose gradient. The RNA was extracted from each fraction and resolved by acrylamide-urea gel electrophoresis and autoradiography. The position at which the 80S monosome sedimented is denoted.

A Gb/B4 RNA carrying a mutation in the U-rich CPE, which prevents polyadenylation, was also examined for its ability to be recruited into polysomes (Fig. 6A, Gb/B4-M2). This RNA was recruited in neither stage VI nor mature oocytes and demonstrates the requirement of polyadenylation for optimal polysomal localization of this mRNA during oocyte maturation.

Previous reports from our laboratory have shown that G10 mRNA recruitment is dependent upon the active process of polyadenylation (14). To determine whether B4 RNA recruitment also requires ongoing polyadenylation, we injected oocytes with a Gb/B4 RNA that already contained a poly(A) tail of about 150 residues, which is the number present on this RNA after maturation (Fig. 6B). Surprisingly, this RNA was translated efficiently in oocytes, with about 50% sedimenting with polysomes. After maturation, this RNA was not further adenylated, nor was more mRNA

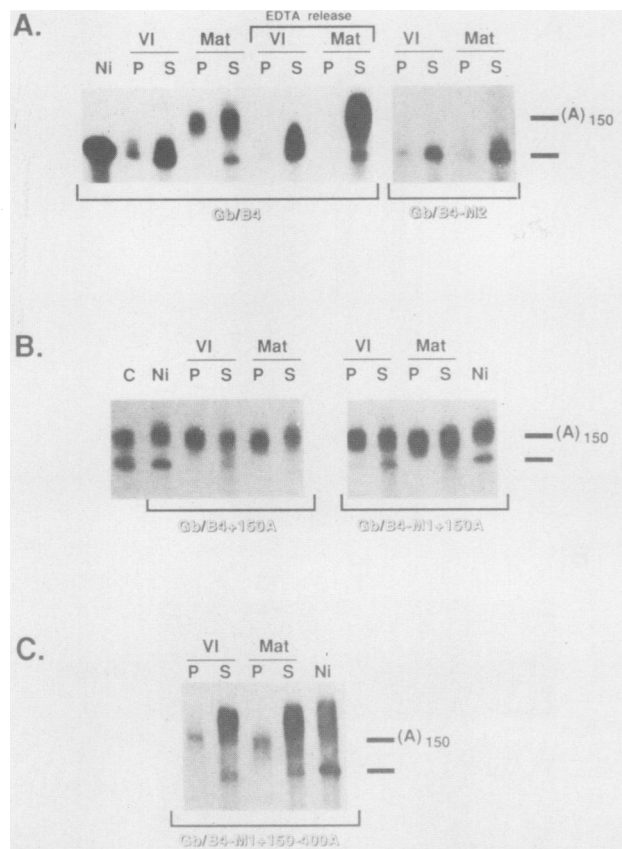


FIG. 6. Recruitment of wild-type and mutant Gb/B4 RNA during maturation. (A) Labeled Gb/B4 or Gb/B4-M2, which has a corresponding *Hind*III site substituted for the U-rich CPE, was injected into oocytes that were matured (Mat) with progesterone. The oocytes were then homogenized, and the homogenate was layered over sucrose and centrifuged. The RNA in the polysomal pellet (lanes P) and postpolysomal supernatant (lanes S) was then extracted and analyzed by electrophoresis and autoradiography as described in the text. The homogenate of some oocytes was also treated with EDTA prior to centrifugation. (B) A poly(A) tail of 150 residues was added to labeled Gb/B4 and Gb/B4-M1 (U-to-G transition in the hexanucleotide) RNAs *in vitro*, and the RNAs were then injected into oocytes. Some oocytes were matured with progesterone, and all oocytes were then homogenized and centrifuged as described for panel A. C (control) refers to Gb/B4 RNA that was polyadenylated during maturation following oocyte injection. (C) Labeled Gb/B4-M1 RNA was polyadenylated *in vitro* with 150 to 400 residues and injected into oocytes. Some oocytes were matured with progesterone, and then all oocytes were centrifuged and analyzed as described for panel A.

recruited into polysomes. Identical experiments were performed on a polyadenylated Gb/B4 RNA carrying a U-to-G mutation in the hexanucleotide sequence (Gb/B4-M1). The behavior of this RNA in stage VI and mature oocytes was indistinguishable from that of wild-type polyadenylated Gb/B4 RNA (Fig. 6B). We therefore conclude from these experiments that B4 RNA, unlike G10 RNA, requires only a poly(A) tail length of about 150 residues to be translated in *Xenopus* oocytes.

The observation that the poly(A) tail of Gb/B4 RNA would not be elongated if it was already of the mature size (i.e., ca. 150 bases) suggested to us that an abnormally long poly(A) tail might have a deleterious effect on the translation of this mRNA. We therefore added a poly(A) tail of 150 to 400

residues to Gb/B4-M1 RNA and assessed whether there is a differential mRNA recruitment based on poly(A) tail size. [We note that although this RNA carries a point mutation in the hexanucleotide sequence, it does not affect poly(A) tail size or translation (cf. Gb/B4-M1 in Fig. 6B).] Interestingly, only the RNA with a poly(A) tail of about 150 residues was translated in stage VI or mature oocytes (Fig. 6C). Therefore, the translational apparatus is unable to recruit an RNA with an abnormally long poly(A) tail.

Polyadenylation in egg extracts. To gain more insight into maturation-specific polyadenylation and its control of translation, we have used extracts from *Xenopus* eggs similar to those used by others for examining cell cycle regulation (17). A Gb/B4 RNA with wild-type *cis* signals was an efficacious substrate for the polyadenylation reaction *in vitro* (Fig. 7A). Similar to what occurs *in vivo*, an RNA with a mutation in the CPE (Gb/B4-M2) was not adenylated and an RNA with a mutation in the hexanucleotide (Gb/B4-M1) was poorly adenylated. This demonstrates that the egg extract does not promiscuously polyadenylate exogenous substrates. Another RNA that was polyadenylated in injected oocytes, Gb/HPRT RNA, was also polyadenylated in the extract, albeit with reduced efficiency (Fig. 7B). Gb/*mos* RNA, which was also adenylated in oocytes, albeit poorly, was not detectably adenylated in the extract (Fig. 7B).

Three lines of evidence suggest that the polyadenylation of different RNAs during maturation may be under at least partially distinct types of regulation. First, the timing of polyadenylation for different RNAs varies during maturation; G10 (McGrew and Richter, *in press*) and Gb/B4 (Fig. 4) RNAs are polyadenylated very early during maturation, whereas D7 RNA is adenylated at a much later time that is coincident with germinal vesicle breakdown (6). Second, the CPE of G10 RNA consists of the dodecamer UUUUUU AUAAAG (McGrew and Richter, *in press*), whereas that of B4 RNA appears to be restricted to the octamer UUUUUAAU (Fig. 3). Third, the poly(A) tail size of B4 RNA is regulated to contain 150 residues; it is not further adenylated if it already contains a poly(A) tail of that size (Fig. 3C and 6B). G10 RNA, on the other hand, is further adenylated at maturation regardless of its initial poly(A) tail size (14). If cellular factors can distinguish between different RNAs (or, specifically, their CPEs), one might expect this to be reflected in competition studies for the polyadenylation machinery. Accordingly, we have performed a series of RNA competition experiments with egg extracts. In each case, the test (tracer) RNA was radiolabeled while the competitor RNA was unlabeled (Fig. 8). For example, unlabeled Gb/B4 RNA was an effective competitor of labeled Gb/B4 RNA for the polyadenylation apparatus. This demonstrates that at least one factor of the polyadenylation machinery is rate limiting. However, although G10 RNA was polyadenylated in the extract, it did not compete at all with labeled Gb/B4 RNA. In the reciprocal experiment, unlabeled Gb/B4 RNA competed efficiently with labeled G10 RNA. Unlabeled XbG10 also competed with labeled XbG10, albeit with somewhat lower efficiency. These data show that G10 RNA is poorly adenylated compared with B4 RNA but that at least one factor is shared between the two RNAs. Such a shared factor has a greater affinity for B4 RNA and denotes the nonequivalency of substrates for the adenylation reaction.

In an attempt to distinguish between putative factors that favor B4 RNA polyadenylation (i.e., those that presumably bind the CPE and hexanucleotide), competition studies were performed with RNAs harboring mutations in the required

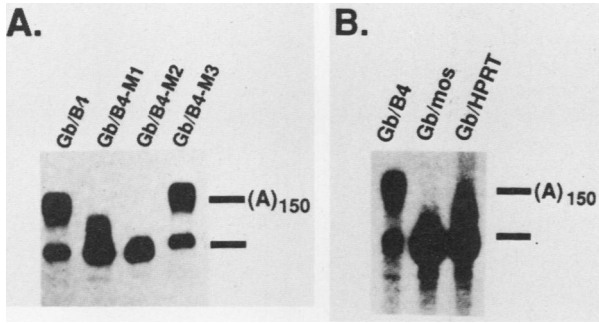


FIG. 7. Polyadenylation in egg extracts. (A) Extracts were prepared from shed eggs and primed with labeled Gb/B4, Gb/B4-M1 (U-to-G transition in hexanucleotide), Gb/B4-M2 (*Hind*III site substituted for U-rich CPE), or Gb/B4-M3 (*Hind*III site substituted for nucleotides immediately 3' of CPE) RNA. Following a 1-h incubation, the RNA was extracted and analyzed by electrophoresis and autoradiography as described in the text. (B) Extracts were primed with labeled Gb/B4, Gb/*mos*, or Gb/HPRT RNA and incubated and analyzed as described for panel A.

cis elements (Fig. 8B). An egg extract was primed with a constant amount of labeled Gb/B4 RNA and excess amounts of unlabeled wild-type B4 RNA or RNAs with mutations in the CPE (M2) or hexanucleotide (M1). Only wild-type Gb/B4 was an efficient competitor. In another extract primed with labeled Xb/G10 RNA and wild-type or mutant Gb/B4 RNAs,

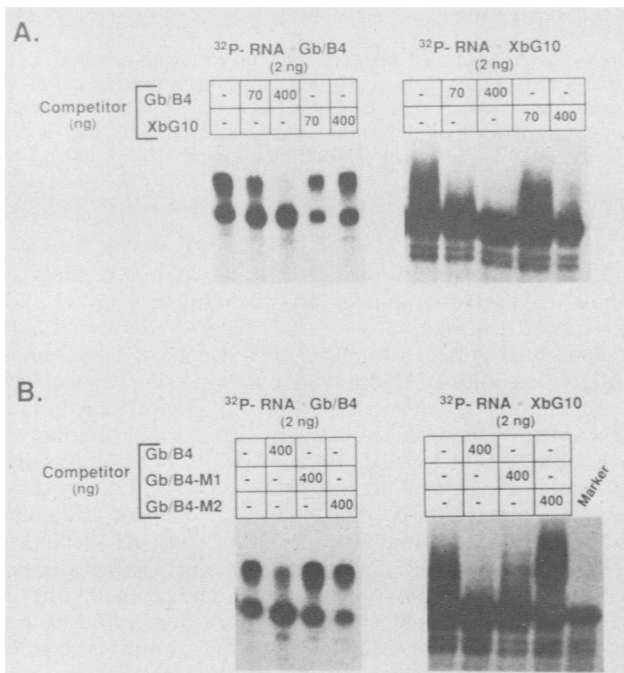


FIG. 8. Competition for polyadenylation in vitro. (A) Egg extracts were primed with the indicated amounts of labeled Gb/B4 or XbG10 RNAs in the absence or presence of the indicated amounts of the same RNAs that were unlabeled. Following incubation, the RNAs were extracted and analyzed as described in the text. (B) Extracts were primed with the indicated amounts of labeled Gb/B4 or XbG10 RNAs in the absence or presence of the indicated amounts of unlabeled Gb/B4, Gb/B4-M1, or Gb/B4-M2 RNAs. Following incubation, the RNAs were extracted and analyzed as described in the text.

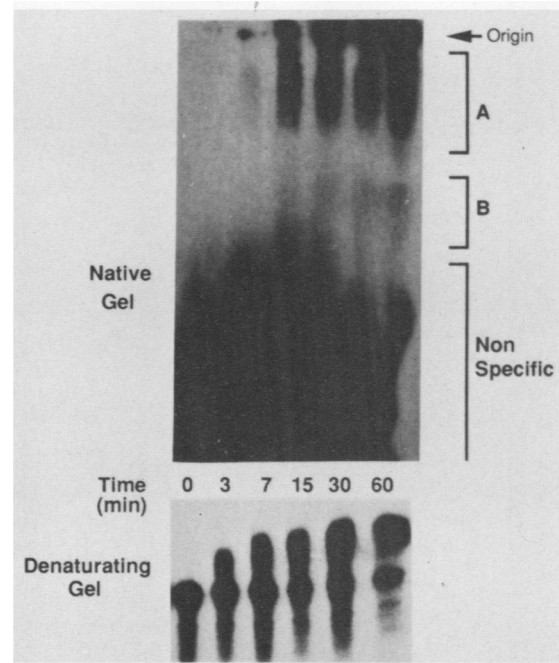


FIG. 9. Timing of polyadenylation complex formation. Labeled Gb/B4 RNA was added to an egg extract, and samples were removed at the times indicated. The RNA from 1/10 of each sample was extracted and analyzed on a denaturing acrylamide gel as described in the text. The remainder of each sample was made to 5 mg of heparin per ml and immediately loaded onto a native 3.5% polyacrylamide gel for electrophoresis and autoradiography. The RNA migrated in three regions of the gel, denoted as A, B, and Nonspecific.

again only wild-type Gb/B4 was an efficient competitor. Such results indicate either that the presumed poly(A) polymerase is rate limiting for polyadenylation or that stable binding of factors to each *cis* sequence occurs only when both are present.

Analysis of polyadenylation complexes. We have used a gel mobility shift assay to examine the polyadenylation apparatus directly. Egg extracts primed with Gb/B4 RNA were analyzed at several time points for polyadenylation complex formation via electrophoresis on a native polyacrylamide gel and for polyadenylation following RNA extraction and resolution on a denaturing agarose gel (Fig. 9). Polyadenylation occurred within 3 min of addition of RNA to the extract and approached completion by 15 to 30 min. Two polyadenylation complexes, denoted A and B, also formed soon after addition of the RNA to the extract, although they were most evident by 7 min. Because maximal complex formation was observed when most molecules were polyadenylated, we conclude that once formed, polyadenylation complexes are stable. Analysis of the RNA in the complexes by electrophoresis in a second denaturing gel revealed that fully adenylated RNA resided in complex A while nonadenylated RNA was present in the nonspecific complex (data not shown). We do not know the polyadenylation status of the RNA in complex B.

To ensure that the protein-RNA complexes we observed were indeed dependent upon the CPE and polyadenylation hexanucleotide, RNAs harboring mutations in these sequences were also tested for their ability to form complexes (Fig. 10A). As a reference, complex formation on wild-type

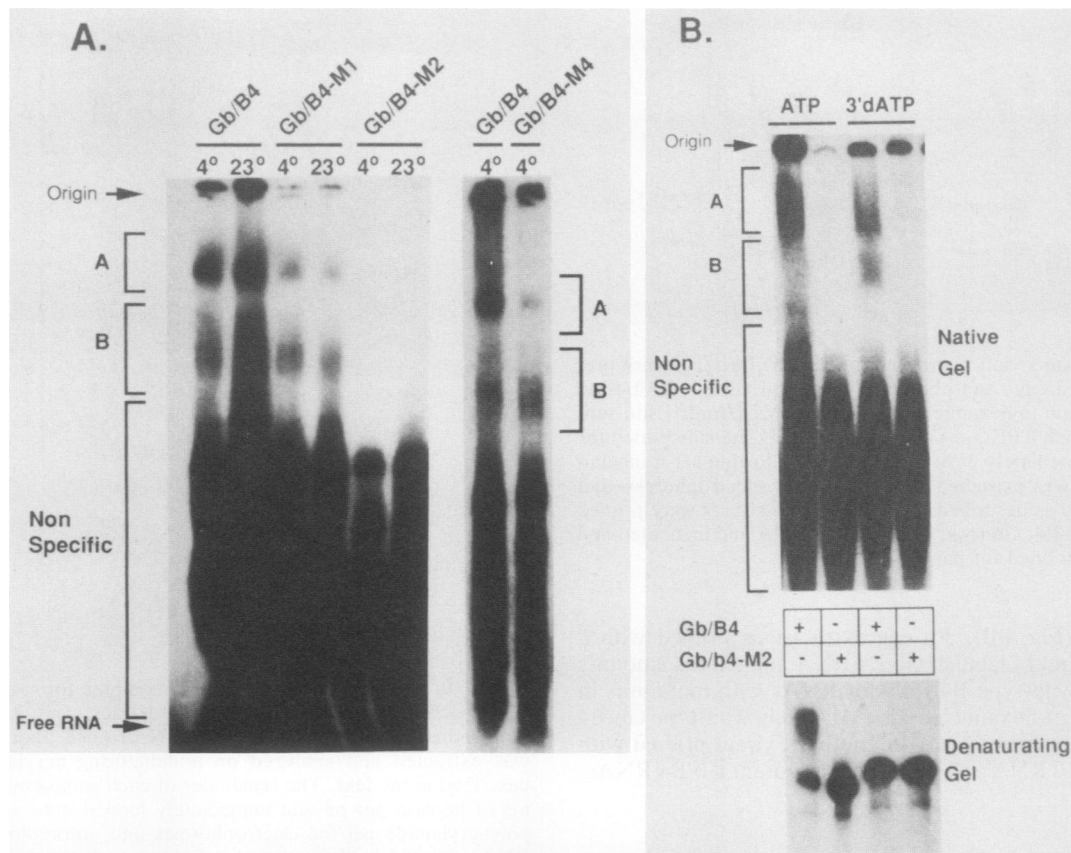


FIG. 10. Analysis of complex formation on wild-type and mutant Gb/B4 RNAs. (A) Egg extracts were primed with labeled Gb/B4, Gb/B4-M1, Gb/B4-M2, or Gb/B4-M2 RNA and incubated for 30 min at 4 or 23°C. The extracts were then made to 5 mg of heparin per ml and immediately loaded onto a native 4% polyacrylamide gel for electrophoresis and autoradiography. (B) Egg extracts were primed with labeled Gb/B4 or Gb/B4-M2 RNAs and incubated for 30 min under standard conditions (1 mM ATP) or in the presence of 5 mM cordycepin (3' dATP). The extracts were then made to 5 mg of heparin per ml and analyzed as described in the text. The RNA from a sample of each extract was also extracted and analyzed for polyadenylation on a denaturing gel.

Gb/B4 RNA was shown to occur at either 4 or 23°C. A small amount of complex also formed on an RNA containing a U-to-G transition in the polyadenylation hexanucleotide (Gb/B4-M1). It should be recalled, however, that B4 RNA contains a second putative hexanucleotide (Fig. 3B) that could conceivably be used in complex formation. Interestingly, mutation of both putative polyadenylation hexanucleotides (Gb/B4-M4) reduced, but did not eliminate, complex formation. Furthermore, an RNA lacking a CPE (Gb/B4-M2) was unable to support any complex formation. Therefore we conclude that specific *trans* factors can bind the CPE independently of the AAUAAA signal but that optimal stable complex formation requires both a CPE and a polyadenylation hexanucleotide.

The next question we have addressed is whether complex formation can occur on RNA substrates that contain both *cis* elements but that cannot be adenylated. Accordingly, cordycepin (3'-deoxyadenosine), which should prevent polyadenylation, was added to an extract in place of ATP. Even though wild-type Gb/B4 RNA was not adenylated in the cordycepin-supplemented extract (denaturing gel), it was still a suitable substrate for complex formation (native gel) (Fig. 10B). For comparison, we show that wild-type Gb/B4 RNA, but not a Gb/B4 RNA lacking a CPE (Gb/B4-M2), was polyadenylated (denaturing gel) and served as a substrate for complex formation in an extract supplemented with ATP

(denaturing gel). In addition, although complex formation occurs at 4°C, polyadenylation is not detected (data not shown). Therefore, ongoing polyadenylation is not required for complex formation.

In a final series of experiments, we have assessed the relative stability of the polyadenylation complex via RNA competitions in egg extracts (Fig. 11). Radiolabeled Gb/B4 RNA was added to an extract with various amounts of wild-type Gb/B4 RNA or B4 RNAs with nucleotide substitutions in the hexanucleotide (M1) or CPE (M2). As shown above, only wild-type Gb/B4 was an efficient competitor when polyadenylation was the assay (Fig. 11, denaturing gel). When complex formation was the assay, wild-type Gb/B4 RNA, but not B4 RNAs with mutations in either *cis* element, was an efficient competitor. Furthermore, complexes A and B were competed equally with wild-type Gb/B4 RNA. We infer from such experiments that at least one component essential for complex formation is rate limiting and that optimal formation of a stable polyadenylation complex requires the interaction of proteins that are bound to each *cis* element.

DISCUSSION

It has recently become clear that polyadenylation has a major influence on the expression of maternal mRNAs

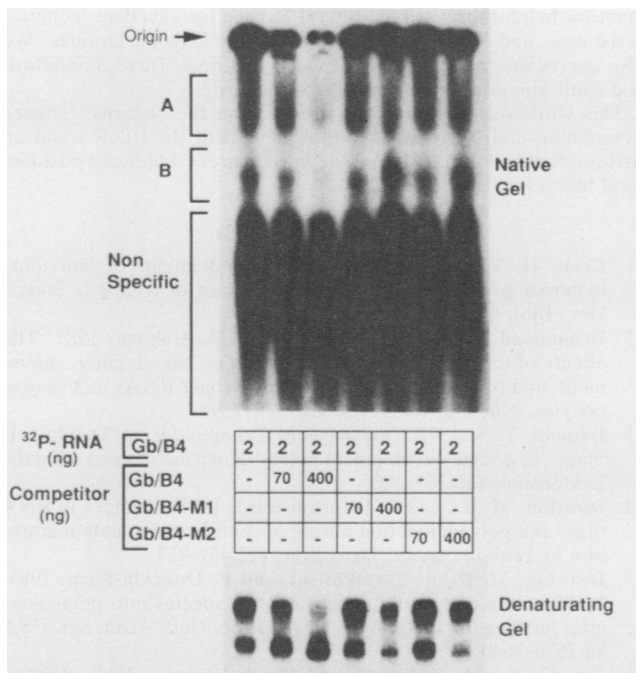


FIG. 11. Competition for complex formation with wild-type and mutant Gb/B4 RNAs. Egg extracts were incubated with the indicated amounts of labeled Gb/B4 RNA in the absence or presence of unlabeled Gb/B4, Gb/B4-M1, or Gb/B4-M2 RNA. Heparin was then added, and the polyadenylation complexes were resolved by native gel electrophoresis and autoradiography. The RNA from 1/10 of each reaction mixture was extracted and analyzed for polyadenylation on a denaturing gel as described in the text.

during oocyte maturation and early embryonic development (reviewed in reference 20). For example, we noted that the translation of G10 RNA during *Xenopus* oocyte maturation was regulated by elongation of its poly(A) tail (14). To gain insight into polyadenylation, we injected in vitro-synthesized RNAs into oocytes and showed a requirement for the *cis* elements AAUAAA and UUUUUUAUAAAG, the latter subsequently being termed the CPE (McGrew and Richter, in press). In this study, we have examined the sequences required for the polyadenylation of other RNAs that we suspected would be polyadenylated during *Xenopus* oocyte maturation. The first RNA, B4, clearly underwent a structural modification during early development and contained what appeared to be a CPE similar to that of G10 RNA (4, 25). Figures 1 and 2 demonstrate that this modification is due to poly(A) elongation that is regulated by the polyadenylation hexanucleotide AAUAAA and UUUUUAAU, which we now define as the B4 RNA CPE. A second RNA, encoding HPRT, is polyadenylated during mouse oocyte maturation (19). This RNA also contained a putative CPE, UUUUAAU, which conferred maturation-specific polyadenylation in injected *Xenopus* oocytes when fused to the 3' terminus of globin mRNA. We do not know, however, whether this chimeric RNA is polyadenylated during mouse oocyte maturation. Finally, we noticed that *c-mos* RNA, which is recruited for translation during *Xenopus* oocyte maturation (23), contained the sequence UUUUAU immediately adjacent to the hexanucleotide AAUAAA. This sequence also confers the polyadenylation phenotype when fused to globin RNA. These data, together with those of Fox et al. (6) and McGrew et al. (14), demonstrate that (i) a CPE

is composed of four or more uridylylate residues closely followed by an invariant AU dinucleotide (although this dinucleotide is not absolutely necessary for polyadenylation [McGrew and Richter, submitted]) and (ii) the CPE can function immediately next to the AAUAAA (Fig. 2), up to 26 bases away (McGrew and Richter, submitted), or either 3' or 5' of the hexanucleotide (6). Although sequence differences between CPEs of different RNAs may be slight, they can have profound effects on the timing and extent of polyadenylation (see below).

Polyadenylation begins soon after progesterone treatment. Progesterone-stimulated oocyte maturation usually occurs over a 5- to 8-h period that is considered complete at germinal vesicle breakdown which is manifested by the appearance of a white spot at the animal pole. B4 RNA polyadenylation is clearly evident by 1 h postprogesterone and thus would be considered an early maturational event. G10 RNA polyadenylation also begins soon after progesterone treatment (McGrew and Richter, in press), whereas D7 adenylation happens at around the time of appearance of the white spot (6). These data therefore show that even though CPEs are similar in sequence, they are regulated in a temporally specific manner.

An interesting point concerning maturation-specific polyadenylation should be noted. B4 RNA can accommodate only 150 adenylate residues; if 150 residues are present initially, no subsequent polyadenylation occurs during maturation (Fig. 3C). This is in contrast to G10 RNA, which is polyadenylated with 100 residues irrespective of the number present initially. How the oocyte senses the presence of a poly(A) tail specifically on B4 RNA is unknown, but the tail appears to be important for the recruitment of this mRNA into polysomes (see below).

mRNA translation is regulated by poly(A) tail length. The data presented in Fig. 6A show clearly that translation of B4 RNA during maturation is regulated by polyadenylation; a mutation in the B4 CPE that inhibits polyadenylation also prevents mRNA recruitment. However, injected B4 RNA that already contains a poly(A) tail of 150 residues is translated efficiently in both stage VI and mature oocytes (Fig. 6B). This demonstrates that poly(A) tail length controls translation of this mRNA, as has been suggested for others (2, 7). Interestingly, the translational apparatus can apparently discriminate against B4 RNAs with an abnormally long poly(A) tail (greater than 150 residues [Fig. 6C]). This underscores our inference that oocytes can sense the poly(A) tail length of B4 RNA (see above) and that this sensation is transduced to the translational apparatus. G10 RNA, on the other hand, requires the process of adenylation for translation regardless of initial poly(A) tail size (14). These two types of translational regulation therefore appear to be fundamentally different responses to progesterone stimulation. It should be noted that this conclusion is based on experiments with chimeric RNAs that consist of the 5' untranslated and coding regions of *Xenopus* B-globin fused to the 3' untranslated region of G10 or B4 RNAs. Therefore, the observed differences in the translational control of these RNAs must reflect the different 3' untranslated regions, or, most probably, their distinct CPEs.

Competition for polyadenylation factors in vitro. To gain insight into the factors involved in maturation-specific polyadenylation, we have used egg extracts that faithfully adenylate exogenously added RNA. Because the polyadenylation hierarchy of RNAs established in vivo (Fig. 2) is maintained in vitro (Fig. 8), we have performed RNA competition studies in an attempt to distinguish factors that

might interact with specific CPEs. Although B4 and G10 RNAs are both adenylated *in vitro*, B4 is by far the better substrate when extracts are challenged with the RNAs simultaneously. This would suggest that at least one factor that is shared by both RNAs has a greater affinity for B4 RNA. However, because each RNA requires two *cis* elements (CPE and hexanucleotide) for adenylation, and therefore probably at least two protein factors (see below), this experiment cannot distinguish which factor is rate limiting. In the hope of examining protein factors individually, we have also used B4 RNAs carrying mutations in each *cis* element. Surprisingly, a mutation in either element renders the RNA competition deficient. From these results, one might infer that the poly(A) polymerase, which is probably shared by G10 and B4 RNAs, is the rate-limiting factor. Alternatively, perhaps factor binding to each *cis* element is greatest when both are present, which would imply an interaction between the *cis* elements via RNA-binding proteins. We favor the later explanation since optimal polyadenylation complex formation requires the CPE and hexanucleotide but not poly(A) polymerase activity (see below).

Formation of polyadenylation complexes. Two polyadenylation complexes are formed in egg extracts challenged with a B4 RNA carrying wild-type *cis* elements. These complexes, which form simultaneously, are stable at 4 or 23°C and are insensitive to treatment with heparin. Optimal formation of complex A in particular requires an intact CPE and a polyadenylation hexanucleotide. Stable complexes are also formed on a wild-type RNA that is not adenylated (in an extract supplemented with cordycepin), which demonstrates that ongoing adenylation is not required for complex formation. Consequently, the observation that efficient competition takes place only when the challenger RNA contains both wild-type *cis* elements indicates that protein binding to each *cis* element is most stable when both are present. As such, we suggest that there is a stable interaction between the proteins associated with the CPE and polyadenylation hexanucleotide. This interaction could be by direct contact or via a third protein intermediate. We also cannot yet eliminate the formal possibility that one factor binds both *cis* elements. However, the data shown in Fig. 2 and others with G10 RNA (McGrew and Richter, *in press*) have shown that polyadenylation occurs even when the *cis* elements are immediately adjacent or far apart. Furthermore, an 82-kilodalton protein photo-cross-links to the G10 CPE but not to the hexanucleotide. Therefore, it seems likely that each *cis* element binds to a distinct protein.

In this communication, we have demonstrated that a U-rich CPE is required for the maturation-specific polyadenylation of three RNAs. Further analysis of one RNA, B4, has shown that polyadenylation occurs very early during maturation and that a poly(A) tail of about 150 residues is required for translation. Competition between B4 and G10 RNAs for polyadenylation activity shows that the RNAs interact with at least one common protein, but that this protein has a stronger affinity for B4 RNA. Finally, we suggest that a stable polyadenylation complex is optimal when there is an interaction of proteins presumed to be bound to each *cis* element. The identification of these proteins requires fractionation of the polyadenylation complex, which we are presently pursuing.

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LITERATURE CITED

- Colot, H. V., and M. Rosbash. 1982. Behavior of individual maternal pA⁺RNA during embryogenesis of *Xenopus laevis*. *Dev. Biol.* **94**:79-86.
- Drummond, D. R., J. Armstrong, and A. Colman. 1985. The effects of capping and polyadenylation on the stability, movement, and translation of synthetic messenger RNAs in *Xenopus* oocytes. *Nucleic Acids Res.* **13**:7375-7394.
- Dumont, J. N. 1972. Oogenesis in *Xenopus laevis* (Daudin). I. Stages of oocyte development in laboratory maintained animals. *J. Morphol.* **136**:153-180.
- Dworkin, M. B., and E. Dworkin-Rastl. 1985. Changes in RNA titers and polyadenylation during oogenesis and oocyte maturation in *Xenopus laevis*. *Dev. Biol.* **112**:451-457.
- Dworkin, M. B., A. Shrutkowski, and E. Dworkin-Rastl. 1985. Mobilization of specific maternal RNA species into polysomes after fertilization in *Xenopus laevis*. *Proc. Natl. Acad. Sci. USA* **82**:7636-7640.
- Fox, C. A., M. D. Sheets, and M. P. Wickens. 1989. Poly(A) addition during maturation of frog oocytes: distinct nuclear and cytoplasmic activities and regulation by the sequence UUUUUU. *Genes Dev.* **3**:2151-2162.
- Gallini, G., E. Kawata, L. D. Smith, and B. A. Larkins. 1988. Role of the 3' poly(A) sequence in translational regulation of mRNAs in *Xenopus laevis* oocytes. *J. Biol. Chem.* **263**:5764-5770.
- Huarte, J., D. Belin, A. Vassali, S. Strickland, and J.-D. Vassali. 1987. Meiotic maturation of mouse oocytes triggers the translation and polyadenylation of dormant tissue-type plasminogen activator mRNA. *Genes Dev.* **1**:1201-1211.
- Hyman, L. E., and M. W. Wormington. 1988. Translational inactivation of a ribosomal protein mRNA during *Xenopus* oocyte maturation. *Genes Dev.* **2**:598-605.
- Konecki, D. S., J. Brennard, J. C. Fuscoe, C. T. Caskey, and A. C. Chinai. 1982. Hypoxanthine-guanine phosphoribosyltransferase genes of mouse and Chinese hamster: construction and sequence analysis of cDNA recombinants. *Nucleic Acids Res.* **10**:6763-6775.
- Krieg, P. A., and D. A. Melton. 1984. Functional messenger RNAs are produced by SP6 *in vitro* transcription of cloned cDNAs. *Nucleic Acids Res.* **12**:7057-7070.
- Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Manley, J. L. 1988. Polyadenylation of mRNA precursors. *Biochim. Biophys. Acta* **950**:1-12.
- McGrew, L. L., E. Dworkin-Rastl, M. B. Dworkin, and J. D. Richter. 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.
- Meinkoth, J., and G. Wahl. 1984. Hybridization of nucleic acids immobilized on solid supports. *Anal. Biochem.* **138**:267-284.
- Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green. 1984. Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmid containing a bacteriophage SP6 promoter. *Nucleic Acids Res.* **12**:7035-7056.
- Murray, A. W., and M. W. Kirschner. 1989. Cyclin synthesis drives the early embryonic cell cycle. *Nature (London)* **339**:275-280.
- Nevins, J. R. 1983. The pathway of eukaryotic mRNA formation. *Annu. Rev. Biochem.* **52**:441-466.
- Paynton, B. V., R. Rempel, and R. Bachvarova. 1988. Changes in state of adenylation and time course of degradation of

- maternal mRNAs during oocyte and early embryonic development in the mouse. *Dev. Biol.* **129**:304–314.
20. Richter, J. D. 1987. Molecular mechanisms of translational control during the early development of *Xenopus laevis*, p. 111–139. *In* J. Ilan (ed.), *Translational regulation of gene expression*. Plenum Publishing Corp., New York.
 21. Rosenthal, E. T., and J. V. Ruderman. 1987. Widespread changes in the translation and adenylation of maternal messenger RNAs following fertilization of *Spisula* oocytes. *Dev. Biol.* **121**:237–246.
 22. Rosenthal, E. T., T. R. Tansey, and J. V. Ruderman. 1983. Sequence-specific adenylations and deadenylations accompany changes in the translation of maternal messenger RNA after fertilization of *Spisula* oocyte. *J. Mol. Biol.* **166**:309–327.
 23. Sagata, N., M. Oskarsson, T. Copeland, J. Brumbaugh, and G. F. Vande Woude. 1988. Function of *c-mos* protooncogene product in meiotic maturation in *Xenopus laevis*. *Nature (London)* **335**:519–525.
 24. Sheets, M. D., and M. P. Wickens. 1989. Two phases in the addition of a poly(A) tail. *Genes Dev.* **3**:1401–1412.
 25. Smith, R. C., E. Dworkin-Rastl, and M. B. Dworkin. 1988. Expression of a histone H1-like protein is restricted to early *Xenopus* development. *Genes Dev.* **2**:1284–1295.
 26. Vassali, J. D., J. Huarte, D. Belin, P. Gubler, A. Vassali, M. L. O'Connel, L. A. Parton, R. J. Rickles, and S. Strickland. 1989. Regulated polyadenylation controls mRNA translation during meiotic maturation of mouse oocytes. *Genes Dev.* **3**:2163–2171.
 27. Wilt, F. H. 1977. The dynamics of maternal poly(A) containing mRNA in fertilized sea urchin eggs. *Cell* **11**:673–681.