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Evolutionary conservation of sequence elements controlling cytoplasmic polyadenylation

(translational regulation/early development/maternal mRNA/polyadenylation/meiotic maturation)

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ABSTRACT Cytoplasmic polyadenylation is an evolutionarily conserved mechanism involved in the translational activation of a set of maternal messenger RNAs (mRNAs) during early development. In this report, we show by interspecies injections that *Xenopus* and mouse use the same regulatory sequences to control cytoplasmic poly(A) addition during meiotic maturation. Similarly, *Xenopus* and *Drosophila* embryos exploit functionally conserved signals to regulate polyadenylation during early post-fertilization development. These experiments demonstrate that the sequence elements that govern cytoplasmic polyadenylation, and hence one form of translational activation, function across species. We infer that the requisite regulatory sequence elements, and likely the trans-acting components with which they interact, have been conserved since the divergence of vertebrates and arthropods.

Key aspects of early development, such as the establishment of body axes and regulation of the cell cycle, often hinge on the regulation of mRNA stability, localization, and translation (1, 2). These forms of control are prominent in the oocyte and early embryo, because the chromosomes at these stages are transcriptionally inactive. Additionally, comparable regulation in somatic cells enables rapid responses to changes in the environment, including perturbations in nutritional, metabolic, and hormonal status.

In development, certain mRNAs are quiescent in the oocyte and become translationally active during late oogenesis or early embryogenesis. Translational activation often is accompanied by, and requires, the elongation of poly(A) tails in the cytoplasm (2–4). Cytoplasmic polyadenylation occurs in a wide range of species, ranging from clam to mouse, indicating that it is a highly conserved mechanism by which mRNAs are regulated during early development. Its biological importance has been shown by experiments with mouse (5) and *Xenopus* c-*mos* mRNAs (6), and with *Drosophila bicoid* mRNA (7).

In mouse and *Xenopus* mRNAs, the 3′ untranslated region (3′ UTR) is necessary and sufficient for cytoplasmic polyadenylation during oocyte maturation. The critical regulatory sequences are referred to as cytoplasmic polyadenylation elements (CPEs) or adenylation control elements (ACEs; 8–11). In *Xenopus*, CPEs have been identified in several mRNAs. They are AU-rich (e.g., UUUUUUAU) and located near the canonical nuclear polyadenylation element, AAUAAA, which also is required for cytoplasmic polyadenylation (3, 8, 9, 12). Detailed mutagenesis experiments establish that minimal perturbations of *Xenopus* CPEs can abolish their function (for review see ref. 3). In the mouse, CPEs also appear to be AU-rich and may resemble their *Xenopus* counterparts (10–12). However, the comparison of putative CPE sequences across species is complicated by the extraordinary AU-richness of 3′ UTRs, and by substantial context and position effects on CPE function (refs. 10 and 13; S.R.T. and M.W., unpublished work). Indeed, the sequence UUUUUAU, a functional CPE in *Xenopus*, is insufficient to cause polyadenylation of certain mRNAs in mouse oocytes (10).

In *Drosophila*, the orchestrated translational activation of maternal mRNAs is necessary for proper development (1, 2). Studies with the anterior determinant, *bicoid* mRNA (14, 15), demonstrated that cytoplasmic polyadenylation is required for its translation (7). The sequence elements that direct poly(A) elongation of *bicoid* mRNA are not yet well defined. However, the 3′ UTR of *bicoid* mRNA is necessary for poly(A) addition and translation because removal of the terminal portion of the 3′ UTR eliminates polyadenylation of injected mRNAs and abrogates their ability to rescue *bicoid* mutant embryos (7).

Although the process of cytoplasmic polyadenylation during early development occurs in many species, the possible conservation of the signals involved has not been tested directly. In this report we examine the conservation of cytoplasmic polyadenylation signals in mouse, *Xenopus*, and *Drosophila* by injecting RNAs derived from one species into the oocytes and embryos of another. Our results establish that the elements that determine whether or not an mRNA will receive poly(A) are functionally conserved, as are those that govern the developmental stage at which the poly(A) is added.

MATERIALS AND METHODS

RNA Sequences and Transcription Templates. *Mouse.* tPA RNA was transcribed from pSP64-Dra (12). It contained the last 455 nt of the 3′ UTR of mouse tPA mRNA plus 32 nt of linker sequence.

Xenopus. Cyclin B1 RNA was transcribed from pGEM −83/+2 cyclin B1 (16). It contained the last 84 nt of the 3′ UTR of cyclin B1 mRNA, plus 10 nt of vector sequence. L1 RNA was generated from pL1/3Z (referred to as L13Z in ref. 17) and contained the last 101 nt of the 3′ UTR of the ribosomal protein L1 mRNA, plus 13 nt of linker sequence. The plasmid encoding L1+CPE RNA (named pL1+CPE/4Z) was constructed using PCR-based site-directed mutagenesis, by inserting TTTTTTAT 8 nt upstream of AATAAA (18) in the pL1/3Z construct, cleaving with *Eco*RI to release the insert, which was then subcloned into pGEM4Z. The plasmid encoding L1+CPE^{mut} (named pL1+CPE^{mut}/4Z) was generated by cleaving pL1+CPE/3Z with *Eco*RI to release the insert, which was subcloned into pGEM3Zf+. Site-directed

Abbreviations: UTR, untranslated region; CPE, cytoplasmic polyadenylation element; tPA, tissue plasminogen activator; CPSF, cleavage and polyadenylation specificity factor; CPEB, CPE-binding protein.
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mutagenesis (19) of pL1+CPE/3Zf+ was performed and the *Eco*RI insert was then transferred to pGEM4Z as above. The sequences of the relevant regions of L1, L1+CPE, and L1+CPE^{mut} RNAs are depicted in Fig. 2.

Drosophila. bicoid RNA was produced from a PCR template generated from pBCD-*Eco*RV (primers used: M13-reverse, GGAAACAGCTATGACCATG and bcd-2, CCCGAGTAGAGTAGTTCTTAT). *bicoid* RNA contained the last 728 nt of the 3′ UTR of *bicoid* mRNA. pBCD-*Eco*RV was derived from pBCD-wt (7) by excising the 5′ UTR and coding region with *Xho*I and *Eco*RV (New England Biolabs); the *Xho*I site was filled-in and the vector religated.

***In Vitro* Transcription.** Capped RNAs of specific radioactivities ranging from 6×10^6 to 4×10^7 cpm/μg were prepared by transcription *in vitro* using bacteriophage RNA polymerases. Transcription reactions were carried out essentially as described by the manufacturers [Epicentre Technologies (Madison, WI)/Promega/BRL]. Transcription reactions were performed using m^{7}GpppG (cap analog) [Ambion (Austin, TX)/New England Biolabs] and 40–140 μCi [α-^{32}P]UTP. All RNAs except those that were injected into mouse oocytes were purified from a gel containing 4–6% polyacrylamide and 7–8.3 M urea prior to injection. For *bicoid* 3′ UTR RNA, if this purification was not performed, poly(A) elongation was not detectable. RNAs were eluted from a gel slice by overnight incubation on an orbital rotator at 25°C or 37°C in 0.1% SDS, 1 mM EDTA, 0.5 M ammonium acetate. The eluted RNA was extracted two to three times with a 1:1 mixture of phenol/chloroform (pH 5.0). In some cases an additional chloroform extraction was performed. The transcript was then precipitated one to three times with 2.5 vol of 100% ethanol. The pellet was washed with 70% ethanol after each precipitation.

The plasmid templates and polymerases used to prepare each RNA substrate were as follows. To prepare mouse tPA RNA, SP6 RNA polymerase (Epicentre Technologies/Promega) was used to transcribe pSP64-Dra that had been linearized with *Xba*I (Promega/Boehringer Mannheim). To prepare *Xenopus* cyclin B1 RNA, T7 RNA polymerase (Epicentre Technologies/Promega) was used to transcribe pGEM-83/+2 cyclin B1 that had been linearized with *Xba*I (Promega/Boehringer Mannheim). To prepare *Xenopus* L1 RNA, T7 RNA polymerase was used to transcribe pL1/3Z that had been cleaved with *Afl*II (New England Biolabs/Boehringer Mannheim). To prepare L1+CPE and L1+CPE^{mut} RNAs, SP6 RNA polymerase (Epicentre Technologies/Promega) was used to transcribe the appropriate DNA templates cleaved with *Afl*II. To prepare *Drosophila bicoid* RNA, T3 RNA polymerase (Epicentre Technologies/BRL) was used to transcribe the PCR-generated template.

Oocyte and Embryo Injections. *Mouse.* Primary oocytes were collected from 3- to 4-week-old BDF1 mice (Taconic Farms), injected, and cultured as described (20–23). Approximately 10 pl of a filtered, 0.1 M KCl solution containing 2.5 pg of RNA were microinjected into the cytoplasm of each oocyte. Injected oocytes were cultured for 16 hr at 37°C in the presence or absence of 100 μg/ml dibutyryl-cAMP (Sigma). (The absence of cAMP stimulates maturation.) Maturation was assessed by the appearance of the first polar body.

Xenopus. Stage VI oocytes were dissected manually from surgically removed ovaries, injected, and incubated essentially as described (8). Approximately 50 nl of a solution containing 2.5–5 fmol of RNA was microinjected into the oocyte cytoplasm. Injected oocytes were incubated at 18°C in 1× Marc's Modified Ringer's (MMR; 100 mM NaCl/2 mM KCl/2 mM $CaCl_2$/1 mM $MgCl_2$/5 mM Hepes, pH 7.4), supplemented with 0.1 mg/ml of penicillin and streptomycin (Sigma). To induce maturation, progesterone (Sigma) was added to achieve a final concentration of 10 μg/ml. Maturation was assessed by the appearance of a white spot on the animal pole of the oocyte.

Eggs were fertilized and dejellied essentially as described (24). To obtain eggs for fertilization, adult females were injected with 50 units of pregnant mare serum (Calbiochem) 2–5 days prior to oviposition. Females were induced to lay eggs by injecting them with 500 units of human chorionic gonadotropin hormone (Sigma). Eggs were collected in MMR at 18°C. For fertilization, eggs were first rinsed with water to remove the MMR. The eggs were then drained of any medium and smeared with macerated testes. Water was then added to activate the sperm. Fertilized eggs were identified by contraction of the animal hemisphere and cortical rotation. To dejelly the fertilized eggs, they were next treated with 2% cysteine (Sigma) in MMR, which had been neutralized to pH 7.8. Eggs were then rinsed several times in MMR and transferred to 5% $Ficoll_{400}$ in MMR. Injection and incubation of the embryos was performed essentially as described (25). Approximately 10 nl of a solution containing 2 fmol of RNA were microinjected into the embryo before first cleavage. Injected embryos were incubated at 18°C in 5% $Ficoll_{400}$ in MMR. At the 64-128-cell stage, the medium was diluted at least 10-fold with water.

Drosophila. Embryos (0-0.5-hr old) were collected and injected anteriorly at room temperature as described (26).

Extraction and Analysis of RNA. *Mouse.* Injected oocytes were homogenized as described (27). The equivalent of 15 oocytes were analyzed on a single lane of a polyacrylamide gel.

Xenopus. Each individual oocyte or embryo was analyzed separately. Single oocytes or embryos were homogenized in 100 μl of 50 mM Tris (pH 7.9), 5 mM EDTA, 2% SDS, and 300 mM NaCl. The homogenate was extracted with a 1:1 mixture of phenol/chloroform (pH 5.0), and the resulting aqueous phase precipitated with ethanol. Each RNA pellet was resuspended in 8 μl of water and 4 μl of loading buffer (28) prior to electrophoresis. The equivalent of 0.5–1 oocyte or embryo was analyzed on a single lane of a polyacrylamide gel.

Drosophila. One hour after injection, *bicoid* RNA was isolated from embryos progressing through development (29). The equivalent of 20 embryos was analyzed on a single lane of a polyacrylamide gel.

Electrophoresis. RNAs were analyzed by electrophoresis through gels containing 4–6% polyacrylamide and 7–8.3 M urea (30).

RNase H/Oligo(dT). The treatment was performed as described (31), using 500 pmol of oligo$(dT)_{15}$ and RNase H from Pharmacia.

RESULTS

Conservation of Cytoplasmic Polyadenylylation Sequences in Mouse and *Xenopus* oocytes. Tissue plasminogen activator (tPA) mRNA is translationally activated by cytoplasmic polyadenylylation during meiotic maturation of mouse oocytes (12, 21). As shown previously, an RNA representing the terminal 455 nt of this mRNA's 3′ UTR (diagrammed in Fig. 1*A*) receives poly(A) during mouse oocyte maturation after injection into the cytoplasm (Fig. 1*A*, lanes 1 and 2) (12). To examine the conservation of regulatory sequences controlling cytoplasmic polyadenylylation, we injected this same RNA into the cytoplasm of *Xenopus* oocytes. Upon injection into frog oocytes, this RNA also receives poly(A) in a maturation-dependent manner (Fig. 1*A*, lanes 3 and 4).

Because a mouse RNA was elongated in frog oocytes, we next performed a reciprocal experiment in which the terminal 3′ UTR of *Xenopus* cyclin B1 mRNA (diagrammed in Fig. 1*B*) was injected into mouse oocytes. As shown previously, this cyclin B1 RNA is efficiently elongated in *Xenopus* oocytes (Fig. 1*B*, lanes 3 and 4), as is the endogenous mRNA (16). In mouse oocytes, injected cyclin B1 RNA also receives poly(A) in the cytoplasm during oocyte maturation (Fig. 1*B*, lanes 1 and 2). The lengths of poly(A) added to both tPA and cyclin B1 are comparable in the two organisms. These experiments strongly

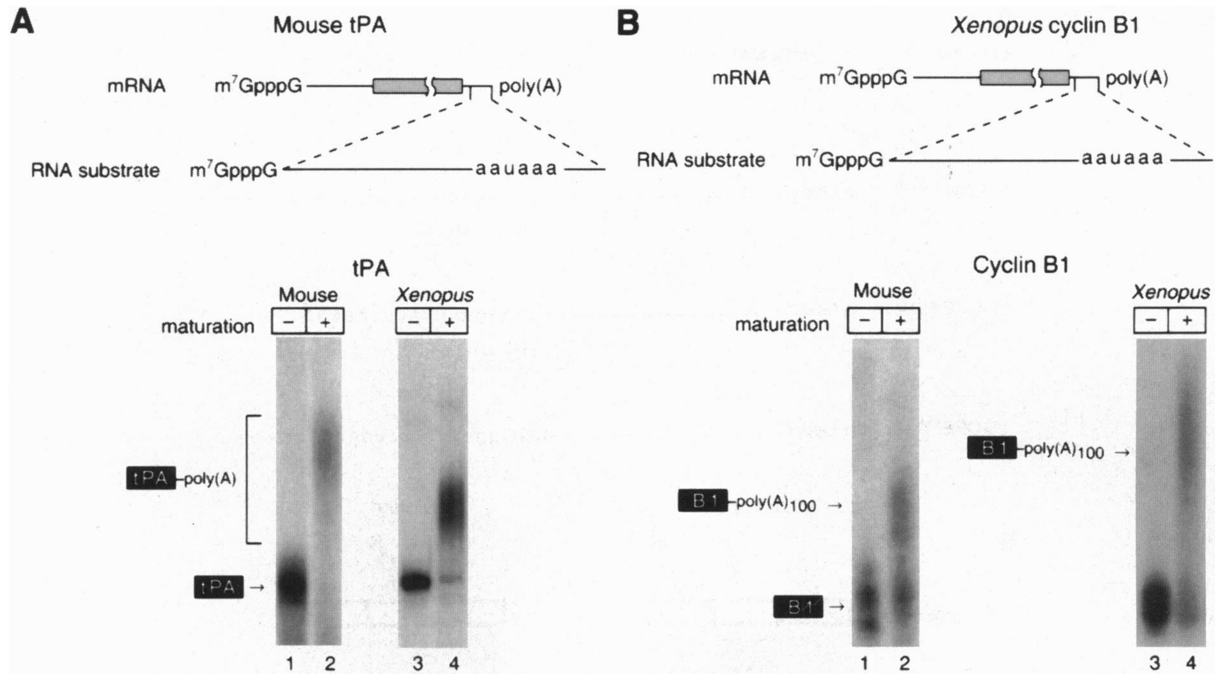

FIG. 1. Polyadenylation of mouse tPA and *Xenopus* cyclin B1 RNAs is properly regulated in interspecies injections. (*A*) (*Top*) Diagram of mouse tPA mRNA and RNA substrate. A filled box represents the coding region and lines represent the 5′ UTR and 3′ UTR. (*Bottom*) Experimental results. tPA RNA was injected into either mouse (lanes 1 and 2) or *Xenopus* (lanes 3 and 4) oocytes. (*B*) (*Top*) Diagram of *Xenopus* cyclin B1 mRNA and RNA substrate. (*Bottom*) Experimental results. Cyclin B1 RNA was injected into either mouse (lanes 1 and 2) or *Xenopus* (lanes 3 and 4) oocytes. In both *A* and *B*, RNA was extracted from oocytes that had (lanes 2 and 4) or had not (lanes 1 and 3) been induced to mature, as indicated above each lane.

suggest that the elements that determine whether or not an mRNA receives poly(A) during oocyte maturation are recognized across species.

To compare more precisely the sequences required for cytoplasmic polyadenylation in mouse and frog oocytes, we injected a series of three RNAs derived from *Xenopus* ribosomal protein L1 mRNA. This maternal mRNA, which does not contain a CPE, is deadenylated during oocyte maturation through a default poly(A) removal activity (18, 32, and A. Bilger, S. Ballantyne, D. Daniel, A. Jenny, and M.W., unpublished work). From ribosomal protein L1 mRNA, we derived three different substrates to be tested for polyadenylation (Fig. 2*A*). L1 RNA is the unaltered wild-type 3′ UTR sequence (the terminal 101 nt of the mRNA); L1+CPE RNA is identical, except that the sequence UUUUUUAU, which has been shown to function as a CPE in *Xenopus* oocytes, has been inserted upstream of AAUAAA; L1+CPE^{mut} RNA contains, in addition to the CPE, clustered base substitutions near the inserted CPE. The results of injecting these "frog" RNAs into *Xenopus* oocytes are shown in Fig. 2*B* (lanes 1–6). The wild-type L1 RNA sequence (L1) did not receive poly(A) during maturation (Fig. 2*B*, lanes 1 and 2), whereas the same RNA into which a CPE had been inserted (L1+CPE) was polyadenylated efficiently (Fig. 2*B*, lanes 3 and 4). The introduction of clustered point mutations near that CPE (L1+CPE^{mut}) reduced polyadenylation efficiency approximately 2-fold, and resulted in the addition of shorter poly(A) tails (Fig. 2*B*, lanes 5 and 6).

The polyadenylation activities of these "frog" RNAs in mouse oocytes closely mimic those observed in the frog. L1, L1+CPE, and L1+CPE^{mut} RNAs were injected into mouse oocytes (Fig. 2*B*, lanes 7–12). As in *Xenopus*, L1 RNA was not elongated (Fig. 2*B*, lanes 7 and 8), whereas L1+CPE RNA was polyadenylated efficiently (Fig. 2*B*, lanes 9 and 10). Polyadenylation of L1+CPE^{mut} RNA in mouse oocytes was attenuated to a degree very similar to that observed in the frog, demonstrating that the similarity observed in vertebrates extended to subtle mutations near the CPE (Fig. 2*B*, lanes 11 and 12). Polyadenylation of L1+CPE and L1+CPE^{mut} RNAs was detected only during oocyte maturation. From these experiments, and those with tPA and cyclin B1, we conclude that the sequences that control cytoplasmic polyadenylation during the meiotic maturation of mouse and *Xenopus* oocytes are similar.

Cytoplasmic Polyadenylation in *Drosophila*. In *Drosophila*, *bicoid* mRNA is translationally activated by cytoplasmic polyadenylation during the first hour after egg activation/deposition (7, 34). The injection of full-length *bicoid* mRNA (≈2.5 kb) into the anterior end of *Drosophila* embryos results in elongation of the transcript by about 150 adenosines (7).

In mouse and *Xenopus* oocytes, synthetic 3′ UTR fragments often exhibit regulated polyadenylation after injection (e.g., Fig. 1). To analyze this property in *Drosophila*, we tested the ability of the terminal 728 nt of *bicoid* mRNA's 3′ UTR (Fig. 3*A*) to become cytoplasmically polyadenylated after injection into fly embryos. This RNA recapitulated the behavior of the full-length mRNA, receiving poly(A) efficiently, as confirmed by RNase H/oligo(dT) treatment (Fig. 3*B*, lanes 1–3). These results show that, as in vertebrate RNAs, signals sufficient for cytoplasmic polyadenylation reside in the 3′ UTR of this *Drosophila* mRNA.

The availability of a short RNA that is elongated in *Drosophila* embryos allowed us to determine whether it would also receive poly(A) in vertebrate embryos. Therefore, we injected the *bicoid* 3′ UTR RNA into *Xenopus* embryos just before the first cleavage division. RNA was recovered from various stages of development, ranging from 2-cell to 5000-cell stage. The injected *bicoid* RNA first became detectably polyadenylated at the 256-cell stage (Fig. 3*C*, lane 2). The polyadenylation increased in the 2000-cell stage (Fig. 3*C*, lane 3), corresponding to early blastula, and was maximal in the 5000-cell, or mid-blastula stage (Fig. 3*C*, lane 4). We conclude that signals in the 3′ UTR of *bicoid* mRNA are sufficient to allow

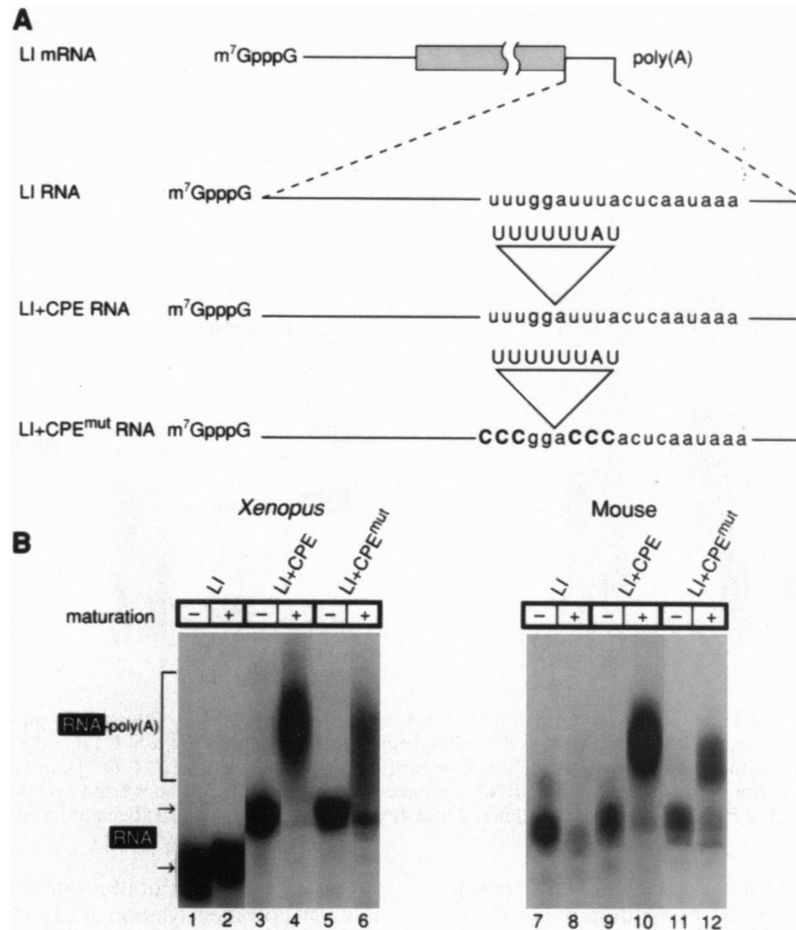

FIG. 2. Conservation of sequence specificity. (*A*) Diagram of ribosomal protein L1 mRNA and RNA substrates. Sequences in the manipulated portion of the L1 sequence are shown. Lowercase letters, wild-type sequence (ending in the polyadenylylation signal "aauaaa"; uppercase letters, inserted nucleotides or base substitutions. (*B*) Experimental results. L1-derived RNAs were injected into *Xenopus* (lanes 1–6) or mouse (lanes 7–12) oocytes. RNA was extracted from oocytes that had (even-numbered lanes) or had not (odd-numbered lanes) been induced to mature, as indicated above each lane.

cytoplasmic polyadenylylation during early embryogenesis in *Xenopus*, as in *Drosophila*.

The *Drosophila* and *Xenopus* embryos used for our injections had been fertilized, resulting in the completion of meiosis, and the onset of mitotic cell cycles and zygotic development. In *Drosophila*, *bicoid* mRNA is not polyadenylylated prior to fertilization (7). Therefore, we examined temporal control of polyadenylylation by injecting *bicoid* RNA into frog oocytes, which are blocked in prophase of the first meiotic division. The injected *bicoid* RNA did not receive poly(A) either in the resting frog oocyte or during oocyte maturation (Fig. 3*D*, lanes 1 and 2). These data suggest that the regulation of the timing of cytoplasmic polyadenylylation is functionally conserved. Similarly, the time after fertilization at which *bicoid* RNA is polyadenylylated in the *Xenopus* embryo is comparable to that observed in *Drosophila* (see *Discussion*). These results suggest that elements that determine the developmental stage at which poly(A) is added function across species.

DISCUSSION

Vertebrates and arthropods diverged approximately 570 million years ago (35). Our results show that, despite this evolutionary distance, the sequences that govern cytoplasmic polyadenylylation during oocyte maturation are functionally conserved between mice and frogs, and the elements that control polyadenylylation after fertilization in *Drosophila* also do so in *Xenopus*. In this sense, cytoplasmic polyadenylylation resembles fundamental cellular processes such as splicing and transcription, in which many of the key sequences, such as splice sites and TATA box promoter elements, are retained during evolution. Although in this report we have analyzed the functional conservation of regulatory elements, our data imply that key trans-acting factors also function across species.

Two observations suggest that the sequences regulating temporal control of cytoplasmic polyadenylylation also are conserved. First, *bicoid* RNA is polyadenylylated after, but not before, fertilization of *Xenopus* embryos. Second, the developmental stage at which injected *bicoid* RNA is polyadenylylated is similar in *Xenopus* and *Drosophila* embryos. In *Drosophila* embryos (7), authentic *bicoid* mRNA receives poly(A) shortly after fertilization, and maintains a maximal poly(A) tail from 30 min ($\approx$8 nuclei) to 2 hr of development, through the syncytial blastoderm stage of 6000 to 8000 nuclei (7). In *Xenopus*, polyadenylylation of injected *bicoid* RNA is first detectable at the 256-cell stage and maximal by the 5000-cell stage. Thus, the developmental stage (i.e., number of nuclear divisions) when poly(A) is added in *Xenopus* substantially overlaps with that in *Drosophila*, though polyadenylylation may begin slightly earlier in the fly. The overlap in temporal control is unlikely to be coincidence, since other RNAs injected into frog embryos receive poly(A) at times that differ substantially from *bicoid* (refs. 36–38; S.R.T. and M.W., data not shown). Similarly, the stages at which poly(A) length is maximal in the two species (syncytial blastoderm and mid-blastula) are very similar with respect to several developmental parameters, such

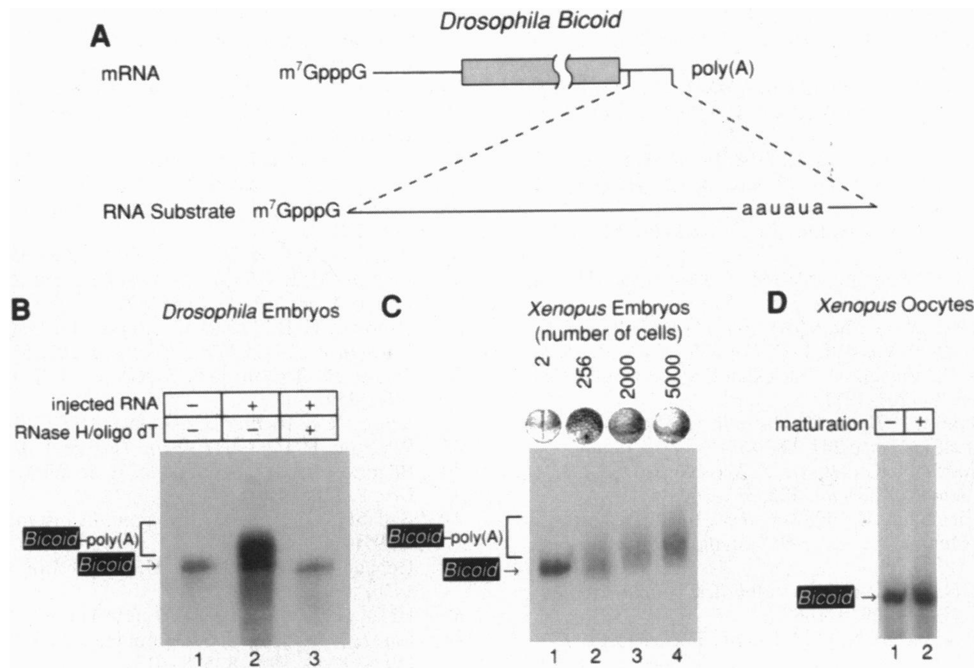

FIG. 3. Polyadenylylation of *Drosophila bicoid* RNA is properly regulated in *Xenopus* oocytes and embryos. (*A*) Diagram of *bicoid* mRNA and RNA substrate. (*B*) *bicoid* RNA injected into *Drosophila*. *bicoid* RNA was injected into the anterior end of *Drosophila* embryos (lanes 2 and 3). RNA extracted from the embryos was either analyzed directly (lane 2) or after treatment with RNase H/oligo(dT) (lane 3). Lane 1 is uninjected *bicoid* RNA. (*C*) *bicoid* RNA injected into *Xenopus* embryos. *bicoid* RNA was injected into 1-cell *Xenopus* embryos, then extracted from embryos containing 2, 256, 2000, and 5000 cells (lanes 1–4). (*D*) *bicoid* RNA injected into *Xenopus* oocytes. *bicoid* RNA injected into stage VI *Xenopus* oocytes, then extracted from oocytes that had (lane 2) or had not (lane 1) been induced to mature.

as the onset of widespread zygotic transcription (39). Although refined mutational analyses will be required to pinpoint the temporal control elements in the two organisms, our results suggest that the elements that determine the developmental stage at which polyadenylylation occurs are recognized across species. *Xenopus* C12 mRNA undergoes cytoplasmic polyadenylylation after fertilization, but not during oocyte maturation (37). For this mRNA, the temporal control arises through two types of elements in its 3′ UTR: one acts negatively, repressing polyadenylylation during oocyte maturation, whereas the other acts positively and is required to activate polyadenylylation after fertilization. Either of these regulatory sequences may be conserved and underlie the stage-specific regulation of *bicoid* mRNA in frog oocytes and embryos.

An extrapolation from our observations is that trans-acting factors required to recognize critical sequences involved in cytoplasmic polyadenylylation are likely to be conserved among vertebrates and arthropods. To date, two well-characterized RNA-binding factors have been implicated in cytoplasmic polyadenylylation: cleavage and polyadenylylation specificity factor (CPSF), a complex of four polypeptides which binds to AAUAAA (17, 40, and A. Bilger, S. Ballantyne, D. Daniel, A. Jenny, and M.W., unpublished work) and CPEB, a CPE-binding protein (41). Both of these factors appear to be conserved. CPSF from calf thymus can functionally substitute for a frog egg RNA-binding activity in catalyzing cytoplasmic polyadenylylation *in vitro*, in fractionated egg extracts (17, 40). This, and related observations (A. Bilger, S. Ballantyne, D. Daniel, A. Jenny, and M.W., unpublished work), imply that the regions of CPSF that interact with the RNA and with crucial components of the frog cytoplasmic polyadenylylation apparatus, such as poly(A) polymerase (41, 42), are conserved. *Xenopus* CPEB (43) is very similar in sequence to the product of the *Drosophila* orb gene, a putative RNA-binding protein required for proper oogenesis in *Drosophila* (33, 44). In *Xenopus*, CPEB and CPSF probably are parts of a core cytoplasmic polyadenylylation apparatus, which acts on many different maternal mRNAs (for review see refs. 2 and 3). Our results suggest that the interactions of these "core" factors with sequences that regulate cytoplasmic polyadenylylation, and perhaps with one another, are conserved during evolution.

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