

Regulated nuclear polyadenylation of *Xenopus* albumin pre-mRNA

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ABSTRACT

Cytoplasmic regulation of the length of poly(A) on mRNA is a well-characterized process involved in translational control during development. In contrast, there is no direct *in vivo* evidence for regulation of the length of poly(A) added during nuclear pre-mRNA processing in somatic cells. We previously reported that *Xenopus* serum albumin [Schoenberg *et al.* (1989) *Mol. Endocrinol.* 3, 805–815] and transferrin [Pastori *et al.* (1992) *J. Steroid Biochem. Mol. Biol.* 42, 649–657], mRNA have exceptionally short poly(A) tails ranging from 12 to 17 residues, whereas vitellogenin mRNA has long poly(A). An RT-PCR protocol was adapted to determine the length of poly(A) added onto pre-mRNA, defined here as that species bearing the terminal intron. Using this assay we show that vitellogenin pre-mRNA has the same long poly(A) tail as mature vitellogenin mRNA. In contrast, albumin pre-mRNA has the same short poly(A) as found on fully-processed albumin mRNA. These results indicate that the short poly(A) tail on albumin mRNA results from regulation of poly(A) addition during nuclear 3' processing.

INTRODUCTION

Nuclear polyadenylation is a complex process involving the ordered assembly of a number of macromolecular complexes (reviewed in 1–4). The highly-conserved polyadenylation signal AAUAAA is the binding site for cleavage and polyadenylation specificity factor, or CPSF, a multisubunit complex consisting of proteins of 160, 100, 73 and (according to one report) 30 kDa (5,6). Sequences in the 3' flanking portion of the pre-mRNA form a complex with cleavage stimulation factor (CstF), another multi-subunit protein consisting of peptides of 50, 77 and 64 kDa (7). The latter peptide binds the U-rich sequence element found in the 3' flanking region of many mRNAs (8). The association of several cleavage factors and poly(A) polymerase complete the known constituents involved in 3' end formation. Cleavage of the pre-mRNA by an as yet unidentified nuclease generates the substrate for polyadenylation.

Poly(A) is added to the cleaved mRNA in two steps: a slow distributive addition of 10 nucleotides (nt) followed by the rapid processive addition of ~250 nt (9). The first reaction requires only CPSF plus poly(A) polymerase (5), whereas the second step is potentiated by the nuclear poly(A)-binding protein II (PAB II) (10). A number of studies have demonstrated a limit of 200–250 residues for nuclear polyadenylation. This upper limit is an inherent feature of the process of nuclear polyadenylation, resulting from the termination of processive poly(A) addition by poly(A) polymerase. In this process the complex of poly(A) with PAB II serves as a molecular ruler to determine the ultimate end of poly(A) addition (11).

The major focus of research in our laboratory is the estrogen regulation of serum albumin mRNA stability in *Xenopus* liver. In male liver albumin mRNA is quite stable, exhibiting a half-life of 8 h (12). Albumin and the other major serum protein-coding mRNAs are destabilized following estrogen administration (13,14) through a pathway that involves the induction of a sequence selective RNase (15,16). In the course of our studies on the regulation of mRNA stability we observed that albumin and several other serum protein-coding mRNAs (transferrin, γ -fibrinogen) had very short poly(A), ranging from 12 to 17 residues (12,14,17). In the case of albumin, poly(A) length was determined by the most rigorous method available: RNase A + T1 digestion of purified mRNA that had been end labeled with [³²P]pCp. There was no effect of estrogen on the length of albumin poly(A), hence destabilization was not coupled to deadenylation as observed with mRNAs like *c-fos* (18,19). We reasoned that the short poly(A) present on albumin and other unstable mRNAs serves to mark these mRNAs for destabilization through the pathway involving the estrogen-induced endonuclease.

The short poly(A) on albumin mRNA could result from two possible mechanisms. The first is cytoplasmic deadenylation, for which there are a number of precedents (18–22). The 17 residues of poly(A) on albumin mRNA is similar to the minimal size limit for poly(A) interaction with the cytoplasmic poly(A)-binding protein PAB I (23), which is distinct from the nuclear PAB II involved in 3' processing. Thus, it seemed likely that albumin mRNA was made with a long poly(A) and was deadenylated to a limit size dictated by binding of (and protection by) PAB I. Alternatively, albumin pre-mRNA might only receive a short poly(A) during nuclear processing. While definitive proof for

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regulation of poly(A) tail length during nuclear pre-mRNA processing is lacking, there are a sufficient number of observations that correlate with this type of mechanism (reviewed in 24) to suggest its feasibility.

The experiments presented here demonstrate that indeed the latter process is responsible for the short poly(A) tail found on albumin mRNA. Our data indicate that albumin pre-mRNA, defined as the species that has both poly(A) and the terminal intron, has the same short poly(A) as the fully processed mature mRNA. To the best of our knowledge this is the first definitive example of regulation of the length of poly(A) added onto nuclear pre-mRNA.

MATERIALS AND METHODS

RT-PCR assay for poly(A)

For analysis of albumin and vitellogenin poly(A) in Figures 1 and 4A first strand cDNA synthesis was performed as described previously (25) on 5 µg of total liver RNA using a 31 nt oligo(dT) primer/adaptor (5'-GCGAGCTCCGCGGCCGGCGT₁₂) as primer. In Figures 2 and 4B a 24 nt primer/adaptor (5'-GGGGATCCGC-GGT₁₂) was used. One tenth of the product was used for each subsequent analysis. Amplification was performed in a 50 µl reaction containing 100 ng each of 5'³²P-end-labeled oligo(dT) primer/adaptor and unlabeled vitellogenin exon 35 primer, 2 mM dATP, dCTP, dGTP and TTP, 2.5 U Vent DNA polymerase in buffer containing 20 mM Tris-HCl, pH 8.8 (at 25°C), 10 mM KCl, 10 mM (NH₄)₂SO₄, 2 mM MgSO₄ and 0.1% Triton X-100 as described by the supplier (New England Biolabs). The reaction mixture was processed through 35 cycles of 94°C/1 min, 60°C/1 min, 72°C/2min, 5 s. The reaction mixture was extracted with an equal volume of phenol:HCCl₃:isoamyl alcohol 25:24:1 and the products were precipitated with ethanol. The resultant pellets were dissolved in water followed by an equal volume of a solution containing 95% formamide, 20 mM EDTA, pH 8.0, 0.1% xylene cyanole and 0.1% bromophenol blue. They were then denatured by heating for 5 min at 95°C and electrophoresed on a 6% acrylamide-8 M urea gel. The dried gel was autoradiographed on Kodak X-Omat XAR-5 or Biomax film. Analysis of vitellogenin poly(A) in Figure 1 was performed with the 31 nt oligo(dT) primer/adaptor and the primer 5'-CACCGCTTGTC-ATGTGAGA located 221 nt 5' to the site of poly(A) addition in exon 35 of the A2 vitellogenin gene. The experiments in Figure 2 and 4B used primers 5'-GTAACCTATAACCAACAATAAC located 110 nt 5' to the site of poly(A) addition in exon 35 of the A2 gene, or 5'-CCAGGCTATAACACATACAG located 360 nt 5' to the poly(A) addition site in intron 34 of the A2 gene. Analysis of poly(A) in the total population of albumin RNA molecules in Figure 2 was performed with the 24 nt oligo(dT) primer/adaptor and the primer 5'-CACTGAGGAACACCTTC-CATCTCT located 118 nt 5' to the site of poly(A) addition in the 74 kDa albumin mRNA and 109 nt 5' to the site of poly(A) addition in the 68 kDa albumin mRNA (26). Analysis of poly(A) in albumin pre-mRNA was performed with the 31 nt oligo(dT) primer/adaptor and the primer 5'-GCCACTGATTCGTATCGA-CAGACC located within intron 14, 405 nt 5' to the site of poly(A) addition.

For the experiment in Figure 1 the product from RT-PCR of vitellogenin mRNA was divided in half. One half was purified on a 2% agarose gel and the other half was divided into two samples, one of which was analyzed directly (lane 1) and the other digested

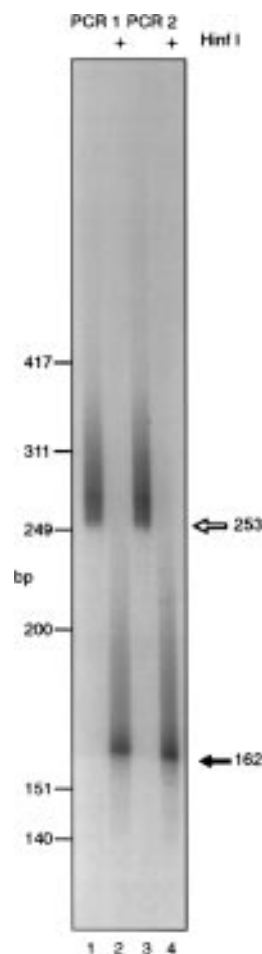


Figure 1. Poly(A) analysis of vitellogenin mRNA by RT-PCR. cDNA prepared to RNA from female *Xenopus* liver was prepared using the oligo(dT) primer/adaptor as primer. Amplification of vitellogenin 3' sequence and poly(A) was performed with a primer 221 nt 5' to the site of poly(A) addition and ³²P-labeled oligo(dT) primer/adaptor. Half of the product was purified on a 2% agarose gel and the remaining material was analyzed directly (lane 1) or digested with *HinfI* prior to gel analysis (lane 2). The gel-purified PCR product was reamplified using the same primers. The product of this reaction was either analyzed directly (lane 3) or digested with *HinfI* prior to gel analysis (lane 4). The open arrow at 252 bp represents the minimal size product expected (221 bp of vitellogenin + 31 bp of primer) for the oligo(dT) primer/adaptor binding adjacent to the site of poly(A) addition. The closed arrow is the minimal size product expected following *HinfI* digestion. The gel was over-run to afford maximal separation of the reaction products.

with *HinfI* prior to gel analysis (lane 2). The gel-purified material was re-amplified as above. Half of this sample was digested with *HinfI* (lane 4) and the other half was not (lane 3). For the experiment in Figure 4A albumin pre-mRNA was amplified with oligo(dT) primer/adaptor (lane 2) and albumin intron 14 primer (lane 8). The products of these reactions were separated on a 2% agarose gel, visualized by ethidium bromide staining, and excised from the gel. The resultant purified DNA was used for a second round of amplification using the oligo(dT) primer/adaptor and albumin exon 15 primer; the former was radiolabeled in the sample in lane 3 and the latter was radiolabeled for the sample in lane 9. These products were extracted with phenol:HCCl₃:isoamyl alcohol, and ethanol precipitated. One third of each reaction was

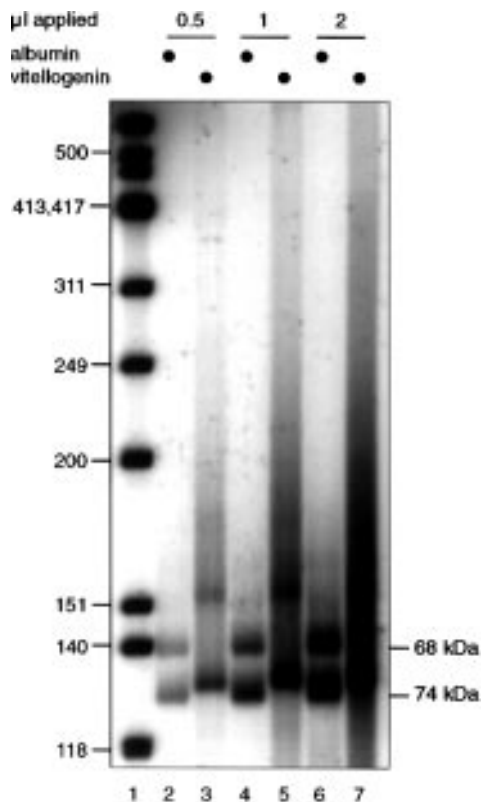


Figure 2. Comparison of RT-PCR assay results for mRNAs with long or short poly(A). cDNA prepared as above was amplified using either a ^{32}P -labeled vitellogenin A2 exon 35 primer 110 bp upstream of the poly(A) addition site (lanes 3, 5 and 7), or a primer to albumin exon 15 located 118 or 109 nt, respectively, upstream of the site of poly(A) addition of the 68 and 74 kDa albumin mRNAs (lanes 2, 4 and 6). The minimal size products expected are 134 bp for the A2 vitellogenin mRNA, 142 bp for the 68 kDa albumin and 133 bp for the 74 kDa albumin mRNAs. Increasing amounts of each RT-PCR product as indicated were loaded in adjacent lanes. Lane 1 contains markers of *HinfI* digested ϕX174 DNA.

either analyzed directly (lanes 3 and 9), digested with *AluI* (lanes 4 and 10), or digested with *PvuII* (lanes 5 and 11).

RESULTS

Validation of the RT-PCR assay for determination of poly(A) length

We previously used two different methods to determine poly(A) length. The first consisted of hybrid selection of a single mRNA species from liver poly(A) mRNA, labeling the 3'-end with [^{32}P]pCp and digestion with RNases A and T1 followed by resolution of the remaining labeled poly(A) on urea/acrylamide gels (12). The second consisted of digestion of the target mRNA in a mixture of total or poly(A) RNA with RNase H in a cocktail containing an oligonucleotide complementary to a sequence 160 nt 5' to the site of poly(A) addition plus or minus oligo(dT). This resulted in the generation of a fragment of 160 nt with or without poly(A) which could then be readily resolved on agarose gels for accurate determination of poly(A) length. These approaches demonstrated that albumin (12), transferrin (17) and γ -fibrinogen mRNAs (14) in *Xenopus* liver all have short poly(A), ranging in length from 17 to 20 nt. In contrast, the estrogen-induced

vitellogenin mRNA has poly(A) of 50–200 nt (17), similar to most eukaryotic mRNAs.

To determine whether the short poly(A) on albumin mRNA resulted from cytoplasmic deadenylation we sought first to determine the length of poly(A) added onto this RNA during 3' processing in the nucleus. Albumin nuclear pre-mRNA is not abundant enough for direct poly(A) determination by 3' end labeling, and for RNase H analysis one would have to hybridize the digestion product with a probe for the last intron. The size of the RNase H digestion product needed for this determination would be sufficiently large that it would preclude analysis of very short poly(A) by Northern blot. We therefore chose to employ a method developed to study cytoplasmic polyadenylation of mRNA in mouse oocytes (27). This assay utilizes an RT-PCR protocol in which reverse transcription is primed with an oligo(dT) primer/adaptor (see Materials and Methods), followed by amplification with this primer and a gene-specific primer located upstream within the body of the mRNA under study. Those authors proposed that the primer/adaptor anneals randomly on the poly(A) tail and the GC-rich portion of the adaptor prevents slipping of the primer on poly(A) during subsequent rounds of amplification.

Since poly(A) lengths of cytoplasmic albumin and vitellogenin mRNA were known from our previous work, these mRNAs were used to qualify the RT-PCR assay for subsequent analysis of albumin pre-mRNA polyadenylation. The experiment shown in Figure 1 used a primer located 221 nt upstream of the site of poly(A) addition of the A2 vitellogenin mRNA (encoded by the terminal exon) in conjunction with the ^{32}P -labeled oligo(dT) primer adaptor. RT-PCR with these primers produced a smear of products ranging in size from 253 to almost 350 nt (lane 1). The minimal size expected was 252 (221 bp of vitellogenin exon 35 plus the 31 bp primer/adaptor). Resolution of species differing by single adenosine residues was obtained by digesting the product in lane 1 with *HinfI*, which cleaves 130 bp 5' to the site of poly(A) addition to yield a minimal product of 162 bp (lane 2). Overall this analysis yielded a preponderance of product adjacent to the site of poly(A) addition that tailed off over a range of 50–60 bp, considerably shorter than the length of vitellogenin poly(A) determined previously (17). Nevertheless this was adequate for the type of analysis needed for the present study. The experiment to determine poly(A) length of albumin pre-mRNA (Fig. 4; see below) necessitated a second round of PCR on a gel-purified product. To insure that subsequent rounds of PCR using the oligo(dT) primer/adaptor did not yield artifactually shorter poly(A) as a result of internal priming, a portion of the mixture remaining from the products shown in Figure 1, lanes 1 and 2 was fractionated on a 2% agarose gel and re-amplified (lane 3). The resultant product was identical to that seen in lane 1. As above, digestion with *HinfI* allowed resolution of individual products differing by single adenosine residues (lane 4).

Having demonstrated that the RT-PCR protocol was capable of measuring poly(A) > 50 residues in length we next sought to determine its utility in detecting the short 17 residue poly(A) tail present on albumin mRNA. In *Xenopus laevis*, genome duplication resulted in four genes for vitellogenin and two for albumin. Based on their degree of sequence relatedness, the vitellogenins are classified A1, A2, B1 and B2. The albumins are classified as 68 and 74 kDa. This latter classification derives from the fact that one of the genes has a single base change introducing a site for N-linked glycosylation (26), which causes the protein product to

migrate more slowly on SDS-PAGE. More relevant to the present study, the divergence of these genes over time has also produced differences in the length of their 3' UTR. For analysis of albumin poly(A) an upstream primer was selected in the terminal exon (exon 15) that hybridizes to both mRNAs, yielding RT-PCR products differing in size by 9 bp. The 68 kDa albumin mRNA should yield a limit product of 142 bp (118 bp 3' UTR + 24 bp primer/adaptor) and the 74 kDa albumin mRNA should yield a limit product of 133 bp (109 bp 3' UTR + 24 bp primer/adaptor). Since the primer/adaptor used in this experiment has 12 thymidine residues and albumin has a 17 residue poly(A) tail the expected result was a defined series of products rather than a long smear. Figure 2 shows a polyacrylamide/urea gel on which increasing amounts of RT-PCR products for albumin or vitellogenin were loaded. For this experiment a primer was selected for A2 vitellogenin mRNA that would yield a product approximately the same size as those anticipated for albumin, so as to enable a side-by-side comparison of the effectiveness of the poly(A) RT-PCR assay on mRNAs with long versus short poly(A). The data in lanes 2, 4 and 6 show that, regardless of the amount of product loaded, RT-PCR of albumin mRNA yields a pair of bands corresponding to the sizes of the products predicted for mRNAs with short poly(A) tails. This contrasted with poly(A) for vitellogenin mRNA (lanes 3, 5 and 7). Like the data in Figure 1, the lowest amount of vitellogenin product shows a strong band at the anticipated size for primer binding adjacent to the site of poly(A) addition, and smearing toward larger species, indicative of long poly(A). As one might anticipate for mRNAs with long versus short poly(A), the intensity of the smear increased with increasing amount of vitellogenin product loaded (lanes 5 and 7), whereas the albumin products retained their discrete character. We conclude from these data that the RT-PCR assay is useful as a qualitative tool to differentiate between short poly(A) and long poly(A).

Albumin pre-mRNA has a short poly(A) tail

Since the amplification performed in Figure 2 used total liver RNA and a primer in albumin exon 15, the products bearing short poly(A) were derived from both unprocessed nuclear pre-mRNA and fully processed cytoplasmic mRNA. Several reports examining the relationship between splicing and polyadenylation of mRNA precursors (28,29) showed that pre-mRNA 3' processing and polyadenylation precede removal of the last intron. Therefore, in the present study albumin pre-mRNA was operationally-defined as that population of polyadenylated (or oligoadenylated) molecules that contain the terminal intron (intron 14).

The experimental scheme employed to measure poly(A) length in intron 14-containing albumin pre-mRNA is shown in Figure 3. Total liver RNA was first reverse transcribed using oligo(dT) primer/adaptor as primer. This was followed by amplification with an upstream primer corresponding to a sequence in albumin intron 14 that should yield a product of 436 bp plus any poly(A) greater than the 31 residues in the primer/adaptor. The product of this reaction was gel purified and a portion was processed through another round of amplification using the 31 nt oligo(dT) primer/adaptor and the exon 15 primer. The second round of amplification was performed with either radiolabeled oligo(dT) primer/adaptor (3' primer) or radiolabeled albumin exon 15 primer labeled (5' primer). This should yield two products with a minimal size of 140 or 149 bp for the 74 and 68 kDa albumin

PCR analysis of poly(A) in intron 14-containing pre-mRNA



Figure 3. Strategy for RT-PCR analysis of poly(A) on albumin pre-mRNA. Albumin pre-mRNA was first amplified by RT-PCR using a primer located within intron 14 located 405 nt 5' to the site of poly(A) addition and the 31 nt oligo(dT) primer/adaptor. This will yield a limit product of 436 [405 bp of albumin + 31 bp of oligo(dT) primer/adaptor]. The product of this reaction was gel purified and split into two portions. The first portion was amplified with the same albumin exon 15 primer as used in Figure 2 and ^{32}P -labeled oligo(dT) primer/adaptor. The second portion was amplified with the same primers, except that the albumin exon 15 primer was ^{32}P -labeled. Digestion with either *Alu I* or *Pvu II* will separate the 5' and 3' portions of the amplified product; size heterogeneity is expected for the 3' portion [detected with the labeled oligo(dT) primer/adaptor], whereas no size heterogeneity is expected for the 5' portion (detected with the labeled albumin exon 15 primer).

mRNAs respectively. The size products generated here will be 7 bp longer than in Figure 2 because of the use of a shorter primer/adaptor in that experiment (see Materials and Methods). Restriction digestion of the RT-PCR product with either *Alu I* or *Pvu II* (which cleave at the same site) should yield a discrete 52 bp fragment from the 5' portion of the amplified products and 88 bp (74 kDa albumin) and 97 bp (68 kDa albumin) products plus added poly(A) from the 3' portion. Reactions were performed with each primer labeled separately to allow unambiguous determination of the length of poly(A) added onto either pre-mRNA.

The results of this approach are shown in Figure 4A. As expected, RT-PCR using either ^{32}P -labeled oligo(dT) primer/adaptor or ^{32}P -labeled albumin intron 14 primer produced a diffuse band of ~450 bp (compare lanes 2 and 8). Reamplification of the gel-purified product in lanes 2 and 8 with either ^{32}P -labeled oligo(dT) primer/adaptor (lane 3) or ^{32}P -labeled albumin exon 15 primer (lane 9) yielded two somewhat diffuse bands at 149 and 140 bp (open arrows). This was identical to the result obtained in Figure 2. Digestion of the product from amplification with ^{32}P -labeled exon 15 primer with either *Alu I* (lane 10) or *Pvu II* (lane 11) yielded the expected 52 bp fragment. In contrast, digestion of the product from amplification with the ^{32}P -labeled oligo(dT) primer/adaptor with *Alu I* (lane 4) or *Pvu II* (lane 5)

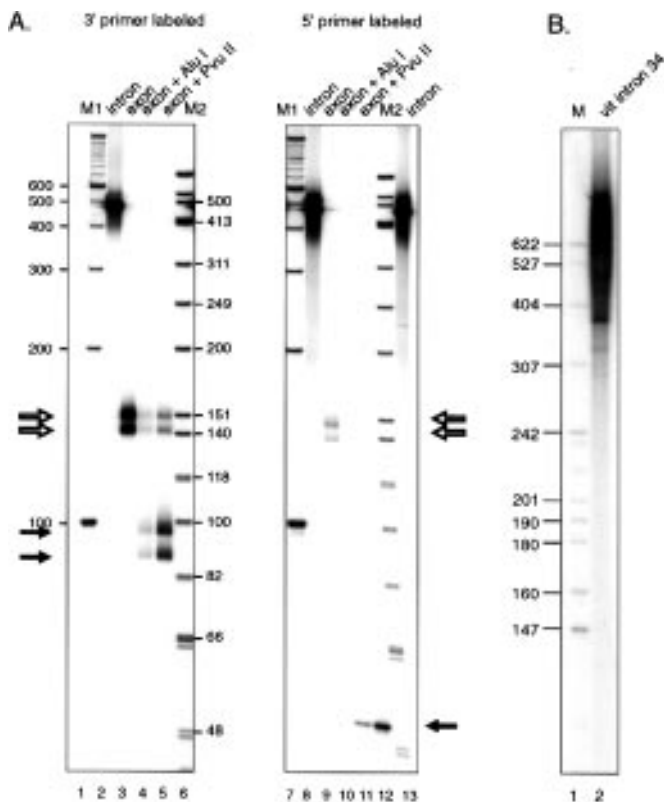


Figure 4. Albumin pre-mRNA has only a short poly(A) tail. (A) Poly(A) on albumin pre-mRNA was analyzed by RT-PCR as described in Figure 3. The products of amplification with either ^{32}P -labeled oligo(dT) primer/adaptor or ^{32}P -labeled albumin intron 14 primer are shown in lanes 2 and 8, respectively. The products of the second round of amplification using either the labeled oligo(dT) primer/adaptor or radiolabeled albumin exon 15 primer are shown in lanes 3 and 9. The open arrows indicate the amplified products from the 68 and 74 kDa albumin pre-mRNAs obtained from this reaction. The results of cleavage of each product from the reactions shown in lanes 3 or 9 with *AluI* or *PvuII* are shown in lanes 4, 5, 11 and 12. The filled arrows indicate the resultant product of the restriction digestions. A small degree of size heterogeneity consistent with short poly(A) was seen only for products amplified with [^{32}P]oligo(dT) primer/adaptor (lanes 5 and 6). The size markers M1 (lanes 1 and 7) are a 100 bp ladder, and markers M2 (lanes 6 and 12) are *HinfI* digested ϕX174 DNA. (B) RT-PCR for poly(A) on vitellogenin pre-mRNA was performed as described in the legend to Figure 2 except that the ^{32}P -labeled upstream primer corresponded to a site 360 nt upstream of the poly(A) addition site within the terminal intron (intron 34, lane 2). The minimal size product expected from this reaction was 384 bp. Lane 1 contains a marker of *MspI*-digested pBR322.

yielded two small groups of bands (filled arrows) corresponding to the 3' UTR + poly(A) of the 68 and 74 kDa albumin mRNAs. A small group of bands differing in size by a single base pair are visible in each product, indicating that intron 14-containing albumin pre-mRNA has the same short poly(A) tail as found in the mature mRNA in the cytoplasm. We conclude from this that the short poly(A) tail on albumin mRNA results from regulation of the length of poly(A) added during nuclear 3' processing and not from cytoplasmic deadenylation. As a final control, RT-PCR analysis was done to evaluate the length of poly(A) on vitellogenin A2 pre-mRNA using a primer to intron 34. The data in Figure 4B show that vitellogenin pre-mRNA has the same long poly(A) tail as seen in Figure 2 using a primer to the terminal exon.

DISCUSSION

Changes in the length of poly(A) in response to extracellular stimuli have been reported for a number of mRNAs. Examples of this include transferrin (17), vasopressin (30,31) and epidermal growth factor (32). However, to date no direct evidence has been reported that alterations in the length of poly(A) on a given mRNA result from regulation of nuclear polyadenylation. In general, most mRNAs receive a long poly(A) tail in what may be considered a constitutive pathway of 3' processing. Albumin mRNA, with its very short poly(A) tail stands in contrast to this.

Clearly, albumin mRNA is transcribed and processed like any other eukaryotic mRNA up to the point of poly(A) addition. Since a *cis*-acting element responsible for regulating the length of added poly(A) remains to be identified we can only speculate at present how a sequence upstream of the 3' processing site can regulate the length of added poly(A). The limited size of albumin poly(A) is similar to the oligoadenylate added in the first step of polyadenylation (9), hence one tempting model is that a sequence in albumin pre-mRNA alters the interaction of polyadenylation factors to effectively prevent the PAB II-stimulated processive addition of poly(A) by poly(A) polymerase (10). While one might dismiss the regulation of poly(A) length as unique to *Xenopus* hepatocytes, preliminary results indicate that we can replicate regulated polyadenylation in transfected LM(tk⁻) and COS-1 cells using an albumin minigene construct (unpublished).

The present study employed an RT-PCR assay that was previously used to study changes in polyadenylation of maternal mRNAs during mouse oocyte development (27). In that earlier report the oligo(dT) primer/adaptor appeared to hybridized randomly on poly(A) prior to reverse transcription, since those authors observed a random distribution of poly(A) lengths consistent with that seen *in vivo*. Before using this assay to examine poly(A) on albumin pre-mRNA we first sought to qualify this assay using as controls mRNAs whose poly(A) length we had determined previously using methods generally accepted to yield accurate poly(A) measurements (12,17). Poly(A) analysis of vitellogenin mRNA by RT-PCR (Fig. 1) gave a size distribution of 12–60 residues, considerably shorter than the 50–200 residues found with the RNase H assay. These findings stand in contrast to the originally published poly(A) RT-PCR assay (27), although subsequent improvements by those authors (33), and the use of a shorter primer/adaptor (Figs 2 and 4B) have overcome this problem.

Lastly, poly(A) has been shown to participate in a number of aspects of mRNA metabolism, and it has been generally accepted that mRNAs with long poly(A) are inherently more stable than mRNAs with short poly(A). In cultured hepatocytes or normal male *Xenopus*, albumin mRNA has a half-life of 8 h. How then, does one account for the stability of this mRNA with such a short poly(A) tail? In yeast the major mRNA decay pathway begins with deadenylation, followed by decapping and 5'–3' degradation by the *XRN1* exonuclease (34). Here, the rate of deadenylation appears to correlate best with relative stability as opposed to the absolute length of the poly(A) tail. There is ample evidence that deadenylation also precedes the degradation of a number of mRNAs in higher eukaryotes. A well-studied example of this is *c-fos* (19,35) from which poly(A) is rapidly removed to oligoadenylate prior to degradation of the body of the mRNA. While we know that the primary turnover pathway in *Xenopus* liver involves induction by estrogen of an RNase selective for

albumin mRNA (15,16), albumin mRNA is likely to also be subject to the constitutive turnover pathways for most mRNAs. This raises the interesting question whether short poly(A) itself is not a feature of instability, but rather for some mRNAs, such as *c-fos*, it may be the process of deadenylation that is ultimately important for their decay. Experiments coupling the albumin poly(A) regulatory element with the elements of *c-fos* involved in deadenylation and destabilization should begin to address this point.

In summary, we report here the first direct evidence for regulation of the length of poly(A) added onto pre-mRNA. Experiments are in progress to determine whether this element is located within the albumin coding sequence or one of the introns of albumin pre-mRNA.

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