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Polyadenylylation of sea urchin histone RNA sequences in transfected COS cells

(*Psammechinus miliaris*/H2A/mRNA processing/pSV2neo/transient expression)

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ABSTRACT **The region of pSV2neo that encompasses the simian virus 40 early polyadenylylation signal was replaced with a DNA fragment that spans the 3′ end of a sea urchin (*Psammechinus miliaris*) histone H2A gene. This clone, pMK2.H2A(3′), was used to transfect COS cells. RNA analysis revealed that transcripts from pMK2.H2A(3′) were polyadenylylated at a site 85 nucleotides downstream from the expected 3′ end of mature H2A mRNA. Nucleotide sequencing showed that the site of poly(A) addition was located 10 nucleotides downstream from a cluster of four A-A-U-A-A-A sequences. The lower accumulation of MK2.H2A(3′) mRNA, which was 5–10% that of SV2neo mRNA, suggests that the H2A polyadenylylation signal is relatively inefficient. The relationship of the above findings to the 3′ end processing of other histone mRNAs is discussed.**

Histone mRNA synthesis differs substantially from that of most eukaryotic mRNAs. Histone gene expression usually is coupled tightly to periods of DNA synthesis and results in transcripts that are 5′ capped but lack internal methylation and are not spliced or polyadenylylated (1–3). The mature 3′ ends of histone mRNAs are highly conserved among species and consist of G+C-rich stem structures followed by the 4 bases A-C-C-A (3). These 3′ ends appear to be generated by the processing of transcripts that extend into 3′ flanking sequences (4, 5).

Unusual histone genes containing introns are found in chickens and *Neurospora crassa* (6, 7). Rare polyadenylylated histone mRNAs are found in HeLa cells (8), chicken erythrocytes (9), and eggs of sea urchins and clams (10, 11). Abundant polyadenylylated histone mRNAs are found in amphibian oocytes (10, 12–15) and in the lower eukaryotes yeast and *Tetrahymena* (16, 17). Common features of many polyadenylylated histone mRNAs are their synthesis in the absence of DNA replication (9–15, 18) and their enhanced stability relative to non-polyadenylylated histone mRNAs (19).

The sequence A-A-U-A-A-A has been implicated in mRNA polyadenylylation (20, 21), either by specifying the site of RNA transcript cleavage (22) or by directing the action of poly(A) polymerase (23). The A-A-U-A-A-A is always found in the 3′ untranslated portion of the mRNA, 5–25 nucleotides upstream from the site of poly(A) addition (24). Histone mRNAs for which sequence information is available* lack the A-A-U-A-A-A sequence in this region.

To investigate processing at the 3′ end of histone mRNA, the polyadenylylation region of pSV2neo (25) was replaced with a DNA fragment that spans the 3′ end of a sea urchin (*Psammechinus miliaris*) histone gene. In sea urchins, this H2A gene directs the synthesis of a mRNA that is not polyadenylylated and exhibits a typical mature 3′ end (26). RNA processing was evaluated after replication and expression of the H2A-containing plasmid in COS cells [simian virus 40 (SV40)-transformed monkey cells]. Analysis of 3′ ends unexpectedly revealed that the resultant H2A-containing transcripts were polyadenylylated. The following report documents the nature of this novel form of mRNA polyadenylylation.

MATERIALS AND METHODS

Plasmids. The construction of pSV2neo [5729 base pairs (bp)] has been described (25) and is shown in Fig. 1*A*. pSV2neoΔ1 (4391 bp) was constructed by deletion of the 1338-bp fragment from pSV2neo (from 4848 to 457) by *Hinc*II–*Hpa* I digestion. pMK2.H2A(3′) is a derivative of pSV2neo that contains, instead of the SV40 early polyadenylylation region, a fragment of sea urchin (*P. miliaris*) DNA that spans the 3′ end of a histone H2A gene [obtained from pBRH2A-3 (27)]. First, pMK2 was constructed: *Xho* I linkers were added to the *Hpa* I site of pSV2neo and the *Hin*dIII site of pUC8 (28). The resultant 36-bp *Xho* I–*Eco*RI fragment of pUC8 that contains poly-cloning sites was exchanged for the 882-bp *Xho* I–*Eco*RI fragment of pSV2neo (deletion from 4848 to 5729). Second, the 934-bp *Xho* I–*Hin*dIII fragment of pBRH2A-3 (just the *Hin*dIII end was blunted by Klenow DNA polymerase) was cloned between the *Xho* I and *Sal* I sites of pMK2 (the *Sal* I site, which is within the poly-cloning region, was also blunt-ended), such that the *Xho* I site was regenerated. The resultant 5800-bp plasmid, pMK2.H2A(3′), contains 240 bp of the 3′ end of the H2A gene (including 188 bp of translated sequences) and 694 bp of sea urchin sequences downstream from the site of the mature 3′ end (Fig. 1*B*).

pSV.H2A contains the complete H2A gene cloned into pSV2neo. The 3092-bp *Hin*dIII–*Eco*RI fragment of pSV2neo (from 2638 to 5729) was replaced by the 1230-bp *Hin*dIII–*Eco*RI fragment of PBRH2A-3. The sea urchin H2A promoter in pSV.H2A is near the *Eco*RI site (27) and directs transcription in an orientation opposite that of the SV40 early promoter.

Transfections. Cultures of COS cells (29) were maintained in Dulbecco's modified Eagle's medium (GIBCO), 10% fetal calf serum (KC Biological, Lenexa, KS), and 5% CO_2 in air at 37°C. Transfections were performed according to the DEAE-dextran procedure of Luthman and Magnusson (30), except the chloroquine treatment was omitted.

RNA Isolation. Total cellular RNA was isolated 35–40 hr following transfection by the lysis method of Chirgwin *et al.* (31) and the centrifugation procedure of Glisin *et al.* (32). Fractionation of total cellular RNA into poly(A)$^+$ and poly(A)$^-$ fractions was performed by oligo(dT)-cellulose chromatography (33).

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Abbreviations: SV40, simian virus 40; bp, base pair(s).

*Genbank, April 1984. (Genbank is the genetic sequence data bank established by Bolt Beranek and Newman, Inc. and Los Alamos Laboratory under contract with the National Institutes of Health.)

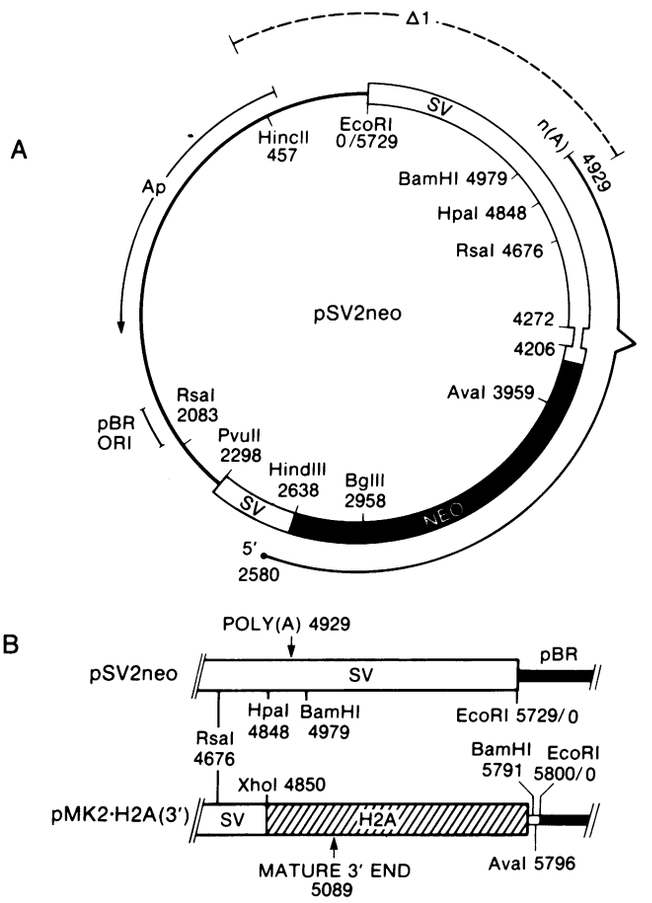

FIG. 1. (*A*) Map of pSV2neo (5729 bp). Sequences are derived from pBR322 (solid line), SV40 (open boxes), and the neo region of Tn*5* (closed box). Numbering begins at the *Eco*RI site. The polyadenylylation site (4929) and intervening sequence (4206–4272) of the transcript that originates from the SV40 early promoter (at 2580) are depicted. pSV2neoΔ1, which lacks the SV40 polyadenylylation site as well as the 5′ end of the β-lactamase gene (Ap) of pBR322, was constructed by deletion of the 1338-bp *Hpa* I–*Hin*cII fragment (dotted line). (*B*) Comparison of pSV2neo and pMK2.H2A(3′). The 882-bp *Hpa* I–*Eco*RI fragment of pSV2neo containing the SV40 early polyadenylylation site was replaced with a DNA fragment containing the 3′ end and flanking region of the sea urchin H2A gene (934 bp, hatched box) and a small region of the poly-cloning site of pUC8 (16 bp, small open box). pMK2.H2A(3′) is 5800 bp. The sites and numbering system for transcription initiation and RNA splicing are identical to those of pSV2neo. The normal 3′ end of mature H2A transcripts (5089) is depicted.

RNA Transfer Blots. RNA samples were denatured by glyoxal, subjected to agarose gel electrophoresis, blotted onto nitrocellulose (Millipore), and hybridized as described by Thomas (34). ^{32}P-labeled probes were prepared by nick-translation (35).

S1 Nuclease Mapping. Restricted DNA fragments were labeled at their 5′ or 3′ ends according to Maniatis *et al.* (36). Probes labeled at only one end were obtained by secondary restriction enzyme digestion and purification by agarose gel electrophoresis. Fragments were recovered from gels by electroelution onto DEAE membranes (NA45, Schleicher & Schuell) (37). Conditions for S1 mapping were as described by Favalaro *et al.* (38). S1 nuclease was obtained from Bethesda Research Laboratories.

DNA Sequencing. The 426-bp *Hae* III–*Dde* I fragment spanning the 3′ end of the H2A gene and extending into the 3′ flanking region was 5′ labeled at the *Hae* III site. Nucleotides were sequenced according to the procedure of Maxam and Gilbert (39).

RESULTS

RNA Transfer Blots. The size and steady-state concentration of RNA transcripts from COS cells transfected with pSV2neo, pSV2neoΔ1, or pMK2.H2A(3′) were compared by RNA transfer blot analysis. Three RNAs were observed in pSV2neo-transfected cells (Fig. 2, left three lanes), all fractionating as poly(A)$^+$ RNA. The relatively weak bands in the poly(A)$^-$ fraction resulted from incomplete binding of poly(A)$^+$ RNA to the oligo(dT)-cellulose. The 2450-nucleotide band is consistent with a RNA having a spliced body of 2283 nucleotides and a poly(A) tail of 150–200 nucleotides (Fig. 1*A*) and is most likely to be SV2neo mRNA. The two less intense bands at 2100 and 1700 nucleotides appear to result from the splicing of additional RNA sequences, for S1 mapping studies revealed bands consistent with an extra 5′ splice junction and two extra 3′ splice junctions in the neo region (unpublished results).

Transfection with pSV2neoΔ1, which lacks the SV40 polyadenylylation site, resulted in the synthesis of heterogeneous transcripts that fractionated exclusively as poly(A)$^-$ RNA (Fig. 2, right three lanes). The identity of the modest band at 4000 nucleotides is unknown. Although 5′ termini were correct (Fig. 5*A*, lane 3), S1 mapping experiments failed to reveal discrete 3′ ends (data not shown). Transcripts prevented from becoming polyadenylylated, therefore, are heterogeneous and accumulate poorly.

Transfection with pMK2.H2A(3′), in which the SV40 poly(A) site was replaced by the 3′ end of a sea urchin histone H2A gene, resulted in the formation of a discrete mRNA of 2700 nucleotides, which fractionated as poly(A)$^+$ RNA (Fig. 2, center three lanes). A non-polyadenylylated RNA of 2443–2509 nucleotides (depending upon whether splicing of the SV40 intron had occurred or not) should have been observed if 3′ end maturation of the H2A sequence had occurred at nucleotide 5089. The appearance of polyadenylylated RNA that was 200–250 nucleotides larger suggests that a poly(A) tail was added at or near the site of the H2A mature 3′ end. In addition to the 2700-nucleotide band, minor bands were apparent that were consistent with the extra splice sites within the neo region.

Location of the Polyadenylylation Site of the H2A-Containing Transcript. S1 mapping experiments of pMK2.H2A(3′)

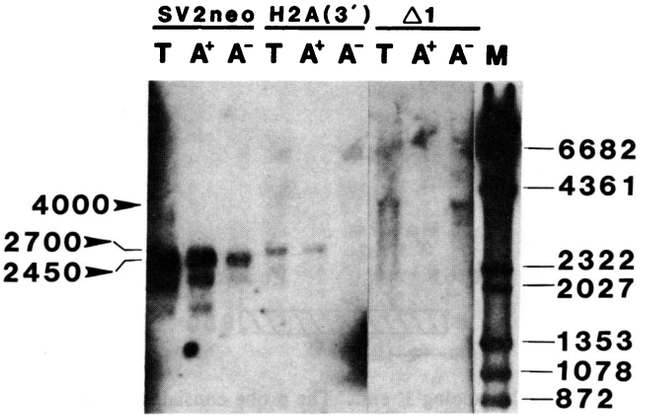

FIG. 2. RNA transfer blots of RNA from transfected COS cells. Total RNA from cells transfected with pSV2neo, pMK2.H2A(3′), or pSV2neoΔ1 was fractionated by oligo(dT)-cellulose chromatography. For each preparation of RNA, 10 μg of total RNA (T), 1.0 μg of poly(A)$^+$ RNA (A$^+$), and 10 μg of poly(A)$^-$ RNA (A$^-$) was subjected to RNA transfer blot analysis. The hybridization probe was specific for neo sequences and consisted of λ DNA that contained a Tn*5* insertion (52). Size markers (M) (shown in nucleotides) were 2 ng of wild-type λ DNA digested with *Hin*dIII. The autoradiograph was exposed for 7 days, with the exception of the marker lane, which was exposed for 1 day.

transcripts were performed by using a 3′ labeled *Xho* I–*Eco*RI (950 bp) fragment of mainly sea urchin DNA as a probe. If poly(A) was added to the site of the mature H2A 3′ end, a band at 240 nucleotides should be apparent. Instead, a single band at 325 nucleotides that fractionated exclusively as poly(A)$^+$ RNA was observed (Fig. 3*A*). This indicates that poly(A) is added to the pMK2.H2A(3′) transcript at a site ≈85 nucleotides downstream from the normal mature H2A 3′ end. These data, together with the RNA transfer blot data of Fig. 2, are consistent with a poly(A) tail length of ≈170 nucleotides.

To confirm that polyadenylylation of the pMK2.H2A(3′) transcripts did not result from an unusual combination of DNA sequences in pMK2.H2A(3′), we constructed a plasmid (pSV.H2A) containing the complete sea urchin H2A gene. In this construct, the orientation of transcription of the H2A sequences is opposite that of pMK2.H2A(3′). S1 mapping of the 3′ ends of pSV.H2A transcripts revealed a 325-nucleotide band identical to that observed for pMK2.H2A(3′) transcripts (Fig. 3*B*).

Sequence of the H2A 3′ Flanking Region. The published *P. miliaris* H2A sequence extended just 27 nucleotides beyond the site of its mature 3′ end.* Since the polyadenylylated 3′ end of MK2.H2A(3′) mRNA maps 85 nucleotides into the flanking region, we sequenced additional bases (Fig. 4). The observed 3′ flanking sequence of the H2A gene is 71% A-T and at a distance 53–74 nucleotides downstream from the normal mature 3′ end, we observed four A-A-T-A-A-A sequences (one of which overlaps two others). Although the redundancy of A-A-T-A-A-As is unusual, the arrangement is consistent with current models for mRNA polyadenylylation, since the site of poly(A) addition in MK2.H2A(3′) mRNA is 10 nucleotides downstream from the furthest A-A-T-A-A-A (Fig. 4). Other noteworthy features of the 3′ flanking sequence were dinucleotide repeats, $(C\text{-}A)_{10}$ and $(C\text{-}T)_{20}$, that occurred downstream from the site of polyadenylylation. $(C\text{-}T)_n$ sequences have been commonly observed in the 3′ flanking sequence of other histone genes (3).

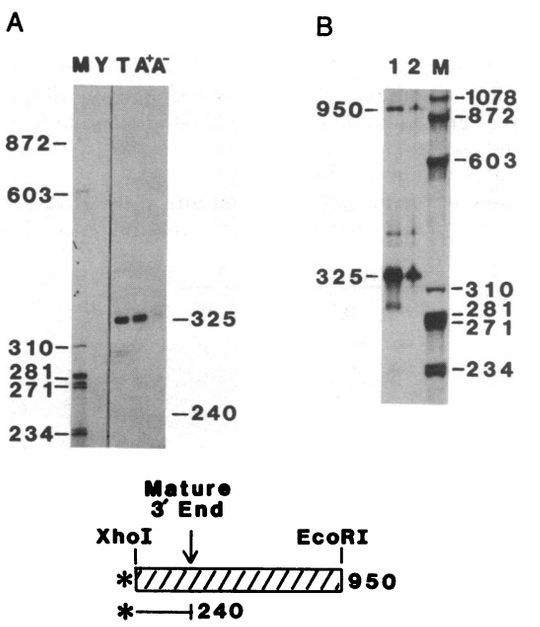

FIG. 3. S1 mapping 3′ ends. The probe consisted of the 950-bp *Xho* I–*Eco*RI fragment of pMK2.H2A(3′) that was 3′ labeled at the *Xho* I site. Hybridizations and S1 nuclease digestions were performed at 52°C and at 30°C, respectively. Size markers (M) (shown in nucleotides) were ϕX174 DNA digested with *Hae* III. (*A*) Analysis of pMK2.H2A(3′) transcripts. The probe was hybridized to 25 μg of yeast tRNA (Y), 10 μg of total RNA from pMK2.H2A(3′)-transfected cells (T), 1 μg of poly(A)$^+$ RNA (A$^+$), or 10 μg of poly(A)$^-$ RNA (A$^-$). (*B*) Comparison of pSV.H2A and pMK2.H2A(3′) transcripts. The probe was hybridized to 10 μg of total RNA from cells transfected with pMK2.H2A(3′) (lane 1) or pSV.H2A (lane 2). The minor bands above and below the 325-nucleotide band disappear upon more extensive S1 nuclease digestion.

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     5070       5080       5090       5100       5110       5120
CTCAACAACGGCCCTTATCAGGGCCACCAAATATTCAAGAAAGATAAAAGTCTCTGQAAT
      ----->    <-----    |
                          mature 3' end

     5130       5140       5150       5160       5170       5180
GAAATCAAGCATCAACAACAAAATAAAATAATAAATAAATAAATAAATATATAAAACAAAAATG
                  ^^^^^^   ^^^^^^   ^^^^^^                 |
                                    ^^^^^^                 poly(A)

     5190       5200       5210       5220       5230       5240
AACAAAGTAATAATAATAGTAAAGAAAATAGACTACCACACACACACACACACACCTC

     5250       5260       5270
TCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT
```

FIG. 4. The 3′ end and flanking sequence of pMK2.H2A(3′). Nucleotides 5060–5116 were previously sequenced by Birnstiel and coworkers (26). The stem structure implicated in 3′ end maturation is indicated by the inverted arrows. The 3′ flanking region begins at nucleotide 5090, which is equivalent to nucleotide +1 of Birchmeier *et al.* (41). The four A-A-T-A-A-A sequences are denoted by carets and the poly(A) addition site is noted.

Map of 5′ Termini and Splice Junctions. S1 mapping experiments comparing the structure of MK2.H2A(3′) mRNA with that of SV2neo mRNA are shown in Fig. 5. The 5′ ends of the mRNAs are identical and map predominantly to nucleotide 2580 (Fig. 5*A*), the expected site of transcription initiation (42). The 66-nucleotide intron from SV40 is accurately processed, since the 5′ and 3′ splice junctions map correctly for both mRNAs (Fig. 5 *B* and *C*). However, the efficiency of splicing differs significantly. For pMK2.H2A(3′) transcripts, the intensity of the band at 791 nucleotides (representative of unspliced RNA) is greater than that of the band at 250 nucleotides (representative of spliced RNA) (Fig. 5*B*, lane 2). In contrast, for pSV2neo transcripts, the intensity of the unspliced (971 nucleotide) band is significantly less than that of the spliced (250 nucleotide) band (Fig. 5*B*, lane 1).

Based on the intensities of the bands in the RNA transfer blot and S1 mapping experiments (particularly the 5′ termini of Fig. 5*A*) the accumulation of MK2.H2A(3′) mRNA appears to be 5–10% that of SV2neo mRNA. Since transcription initiated from identical promoters, the lower accumulation of MK2.H2A(3′) mRNA probably results from the relative inefficiency of its polyadenylylation signal. The data are consistent with 5–10% of the pMK2.H2A(3′) transcripts being polyadenylylated and the polyadenylylated RNAs accumulate while the non-polyadenylylated ones degrade. The efficiency of polyadenylylation of the pSV2neo transcripts is likely to be 10- to 20-fold higher.

DISCUSSION

We have found sea urchin histone H2A-containing transcripts to be polyadenylylated in COS cells at a site that is 85 nucleotides downstream from the site of the 3′ end of normal sea urchin mRNA.* The hexanucleotide sequence A-A-U-A-A-A appears to be involved, since repeats of this sequence occur 10 bases 5′ to the site of polyadenylylation. Although A-A-U-A-A-A is required for the polyadenylylation of most mRNAs, this sequence alone is not sufficient (21). The importance of other nearby sequence elements has been recognized (43–45). It is likely that the redundancy of the A-A-U-A-A-A sequences may contribute significantly to the extent of H2A polyadenylylation, because of the lack of obvious neighboring features.

The aberrant 3′ end maturation of sea urchin H2A transcripts observed in our experiments is not unique to COS cells. It also has been seen in HeLa cells that were transfect-

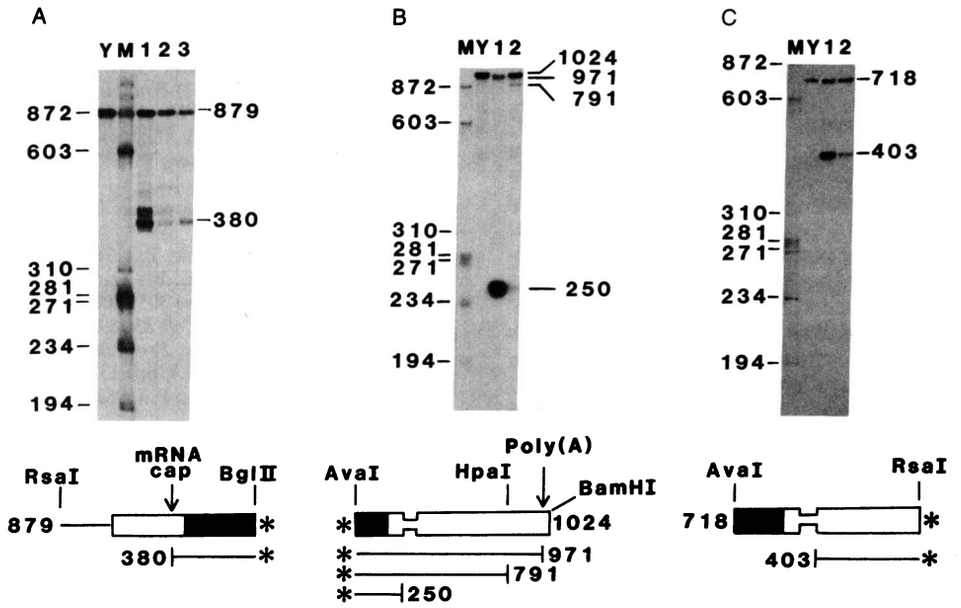

FIG. 5. S1 mapping 5′ ends and splice junctions. End-labeled probes were hybridized to 25 μg of yeast tRNA (Y) or to 10 μg of total RNA from cells transfected with pSV2neo (lanes 1), pMK2.H2A(3′) (lanes 2), or pSV2neo 1 (lanes 3). Markers (M) (shown in nucleotides) were φX174 DNA digested with *Hae* III. (*A*) Mapping the 5′ end. The probe consisted of the 879-bp *Rsa* I–*Bgl* II fragment of pSV2neo, which spans the SV40 promoter region and was 5′ labeled at the *Bgl* II site. Hybridization was performed at 52°C. (*B*) Mapping the 5′ splice junction. The probe consisted of the 1024-bp *Ava* I–*Bam*HI fragment of pSV2neo, which spans the intervening sequence and was 3′ labeled at the *Ava* I site. Hybridization was performed at 52°C. Spliced SV2neo and MK2.H2A(3′) transcripts protected the same 250-nucleotide band. Unspliced transcripts from pSV2neo protected a band at 971 nucleotides (the band in this region in lane 1 is actually a closely spaced doublet that consists of re-annealed probe and the 971-nucleotide band). Unspliced transcripts from pMK2.H2A(3′) protected a smaller band of 791 nucleotides, because the probe is homologous with pMK2.H2A(3′) only up to the *Hpa* I site. (*C*) Mapping the 3′ splice junction. The probe consisted of the 718-bp *Ava* I–*Rsa* I fragment of pSV2neo, which spans the intervening sequence and was 5′ labeled at the *Rsa* I site. Hybridization was performed at 42°C.

ed with a plasmid containing the h22 sea urchin histone cluster (46). An S1 mapping experiment of the 3′ ends of the resultant H2A transcripts in HeLa cells revealed the same 325-nucleotide band observed in our COS cell experiments.

In contrast to the transfection of HeLa and COS cells, injection of sea urchin histone H2A DNA into *Xenopus laevis* oocytes resulted in the synthesis of H2A mRNAs with normal, mature 3′ ends (41). Similarly, injected oocytes directed the synthesis of chicken H2B mRNAs with normal mature 3′ ends (5). Possible explanations for the disparate results are that histone mRNA maturation may be mediated by RNA processing factors, such as small nuclear ribonucleoproteins (47), that exhibit a limited phylogenetic range or exhibit a defined temporal or spatial availability. The latter explanation is attractive, because histone mRNA synthesis in typical eukaryotic cells, unlike that of oocytes, is coupled tightly to DNA synthesis (2, 3). pSV2neo-derived plasmids replicate autonomously in COS cells to high levels within the short time span (35–40 hours) of the transfection experiments described in this report (29). Thus, the unavailability of appropriate RNA processing factors outside of S phase may have resulted in the failure of COS cells to mediate normal 3′ end maturation of the sea urchin H2A RNA sequences. Accordingly, polyadenylylation in COS cells at the site downstream from the mature sea urchin H2A 3′ end may have occurred by default.

Deletion analysis has shown that 80 nucleotides of 3′ flanking sequence are essential for correct 3′ end maturation of sea urchin H2A mRNAs (41). Deletion of additional nucleotides would result in the loss of the A-A-U-A-A-A sequences that are implicated in polyadenylylation. This raises the formal possibility that cleavage and/or polyadenylylation at the site 85 nucleotides into 3′ flanking sequence may be an intermediate step in the normal 3′ end maturation of sea urchin H2A mRNA.

Precise sites for termination of histone gene transcription are not known (3) and no direct evidence for discrete terminator sites for sea urchin H2A transcripts was obtained in our experiments, even when probes spanned the entire 3′ spacer region. These data suggest that termination in COS cells within the sea urchin spacer region may be extremely heterogeneous or result in transcripts that are rapidly degraded.

Polyadenylylation of Other Histone mRNAs. Although usually rare, polyadenylylated histone mRNAs are abundant in certain cells. Up to 75% of the histone mRNA is estimated to be polyadenylylated in *X. laevis* oocytes (13–15). Since histone mRNAs lack A-A-U-A-A-A sequences in 3′ untranslated regions, the identity of the signals directing the synthesis of polyadenylylated histone mRNAs is unclear. Poly(A) polymerase may possibly add poly(A) tails directly onto mature 3′ ends in an A-A-U-A-A-A-independent fashion. Sequence analysis of three *Xenopus* H4 cDNA clones constructed via oligo(dT) priming is consistent with this possibility (48, 49). Polyadenylylation of histone transcripts may alternatively depend upon the recognition of A-A-U-A-A-A sequences found downstream of the normal mature 3′ ends. The histone transcripts polyadenylylated by this mechanism would be expected to contain, like the H2A transcripts described in this report, additional 3′ flanking sequences. Experiments designed to map the poly(A) addition sites of other polyadenylylated histone mRNAs would differentiate between the two possibilities.

The probability of finding A-A-U-A-A-A sequences downstream from the site of normal, mature histone 3′ ends is high. Spacer regions between histone genes of most species are often 1 kilobase or more and A+T-rich (3). Assuming the spacer is 60–70% A-T, it is probable that an A-A-U-A-A-A will occur randomly each 544–1372 bases. Of 17 histone genes, for which at least 100 bp of 3′ flanking sequences are

known, 7 have an A-A-U-A-A-A in their 3′ flanking region: chicken H2B, *X. laevis* H1, yeast H2A, *Strongylocentrotus purpuratus* H2B, *Lytechinus pictus* H4, and *P. miliaris* H4 and H2A.*

Polyadenylylation of certain histone transcripts at 3′ flanking sequence sites is likely if normal 3′ end maturation is inhibited. During early oogenesis in amphibians, the time of synthesis and accumulation of polyadenylylated histone mRNAs (50), mature 3′ end formation generally is aberrant. This alteration in processing results in the synthesis of transcripts that are larger than those produced at other stages and may contain interspersed repeats (40, 51). Aberrant 3′ end maturation could be due to the lack of synthesis or availability of certain small nuclear ribonucleoproteins (47) or other factors at this early stage of oogenesis. The 3′ end maturation factors may be available only during periods of DNA synthesis. This would explain why the synthesis of many polyadenylylated histone transcripts is uncoupled from DNA replication (9–15, 18). However, the availability of 3′ end processing factors is not necessarily limited only to periods of DNA synthesis. Such factors are undoubtedly present during later stages of *Xenopus* oogenesis, since oocytes injected at these stages demonstrate the capacity to synthesize histone mRNAs that are not polyadenylylated and have mature 3′ ends.

Our analysis of the 3′ ends of histone transcripts in transfected COS cells raises questions concerning the identity of the sites at which histone transcripts may be polyadenylylated. Additional RNA mapping studies are needed to document the nature and possible regulation of processing events that occur at the 3′ ends of RNA polymerase II transcripts.

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