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An Intact Histone 3′-Processing Site Is Required for Transcription Termination in a Mouse Histone H2a Gene

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A transcription termination site has been characterized between the mouse histone H2a-614 and H3-614 genes. There is a poly(A)$^-$ RNA present in small amounts in the nucleus which ends 600 nucleotides 3′ to the H2a-614 gene. Nuclear transcription studies demonstrate that transcription extends at least 600 nucleotides 3′ to the gene but is greatly reduced 700 nucleotides 3′ to the gene. If all or part of the normal 3′-processing signal, consisting of the stem-loop and the U7 small nuclear ribonucleoprotein binding site, is deleted, transcription then continues past the putative termination site and RNAs which end at the 3′ end of the downstream H3-614 gene accumulate. Insertion of a 150-nucleotide fragment containing the termination site between the histone 3′ end and downstream polyadenylation sites reduces usage of polyadenylation sites 85 to 90%. Taken together these results suggest there is a transcription termination site which requires an intact histone 3′-processing signal to function.

Histone mRNAs are the only major class of mRNAs transcribed by RNA polymerase II which is not polyadenylated. The 3′ end of histone mRNA in higher eucaryotes is formed by an endonucleolytic cleavage (15, 37) after a stem-loop sequence. There is a purine-rich sequence 10 to 20 nucleotides (nt) 3′ to the stem-loop, which binds U7 small nuclear ribonucleoprotein (snRNP) and is required for 3′-end formation (32, 36). RNA polymerase II presumably terminates at some point downstream of the 3′-processing signal. The specific sites of transcription termination (indeed, whether specific transcription termination sites exist) have remained elusive. There is convincing evidence that RNA polymerase II often proceeds at least several hundred nucleotides 3′ to the gene before terminating (10, 11, 20).

Transcription termination sites are not coincident with the 3′ ends of mature RNAs except for the RNAs synthesized by RNA polymerase III and possibly also the snRNAs transcribed by RNA polymerase II (23, 39). Transcription termination sites have been studied in a number of genes which encode polyadenylated mRNAs (43). It has been suggested that in some cases transcription termination occurs at multiple sites in a stretch of DNA extending over hundreds of nucleotides (20). Citron and co-workers have suggested that transcription of the β-globin gene terminates heterogeneously within 1,000 bases downstream of the globin gene (11). There is convincing evidence for a number of genes that transcription termination requires the presence of a functional polyadenylation site (12, 27, 31, 47). Thus, removal of the 3′-processing signal resulted in continued transcription past a termination site which functioned when the processing signal was present (47).

The mechanism of transcription termination is less clear for histone genes. Sequences directing transcription termination in histone genes were first investigated in the sea urchin histone H2a gene by Birchmeier and co-workers (4). They suggested that termination of transcription in the sea urchin H2a gene injected into *Xenopus* oocytes occurred about 200 nt downstream of the gene. This same sequence has been shown to terminate transcription of heterologous genes in mammalian cells in the absence of a 3′-processing signal (8, 25), suggesting that the mechanism of transcription termination may differ between histone genes and genes encoding polyadenylated mRNAs.

There is a pair of mouse histone genes, H2a-614 and H3-614, which are transcribed from the same strand of the DNA and are only 800 nt apart (17). Both these genes are expressed at very high rates. The downstream gene in this cluster, H3-614, has its own promoter (29, 46). It is very likely that transcription termination occurs between these two genes. We have detected a sequence which by a number of criteria may be a transcription termination site. This termination site requires an intact 3′-processing signal to function and is located within the sequences required for high-level expression of the H3.2 gene.

MATERIALS AND METHODS

Cell growth and transformation. Mouse L cells (thymidine kinase negative) were grown and exogenous DNA was introduced by cotransfection with the herpes simplex virus thymidine kinase gene as previously described (1). Chinese hamster ovary (CHO) cells were grown in alpha media supplemented with 10% fetal calf serum, and exogenous genes were introduced by cotransfection with the neomycin phosphotransferase gene by the Polybrene method of Chaney et al. (9) as previously described (29). The transfected cells were selected by growth in G418 (1.5 mg/ml) for 1 week, followed by growth for 10 days in 400 μg of G418 per ml. Pools of 30 to 50 stable transfectants were used in all experiments.

DNA subclones. The following DNAs were used in the transfection experiments. The modified H2a-614 genes are shown in Fig. 1A. (i) A 3.1-kb *Eco*RI-*Hin*dIII fragment from the MM614 fragment containing the entire H2a-614 and H3-614 genes (17) was the parent clone used in these experiments (Fig. 1A). The sequence of this fragment, extending from 850 nt upstream of the start of the H2a-614 protein to 470 nt 3′ of the end of the H3-614 mRNA, has been previously reported (24, 46). Two constructs containing

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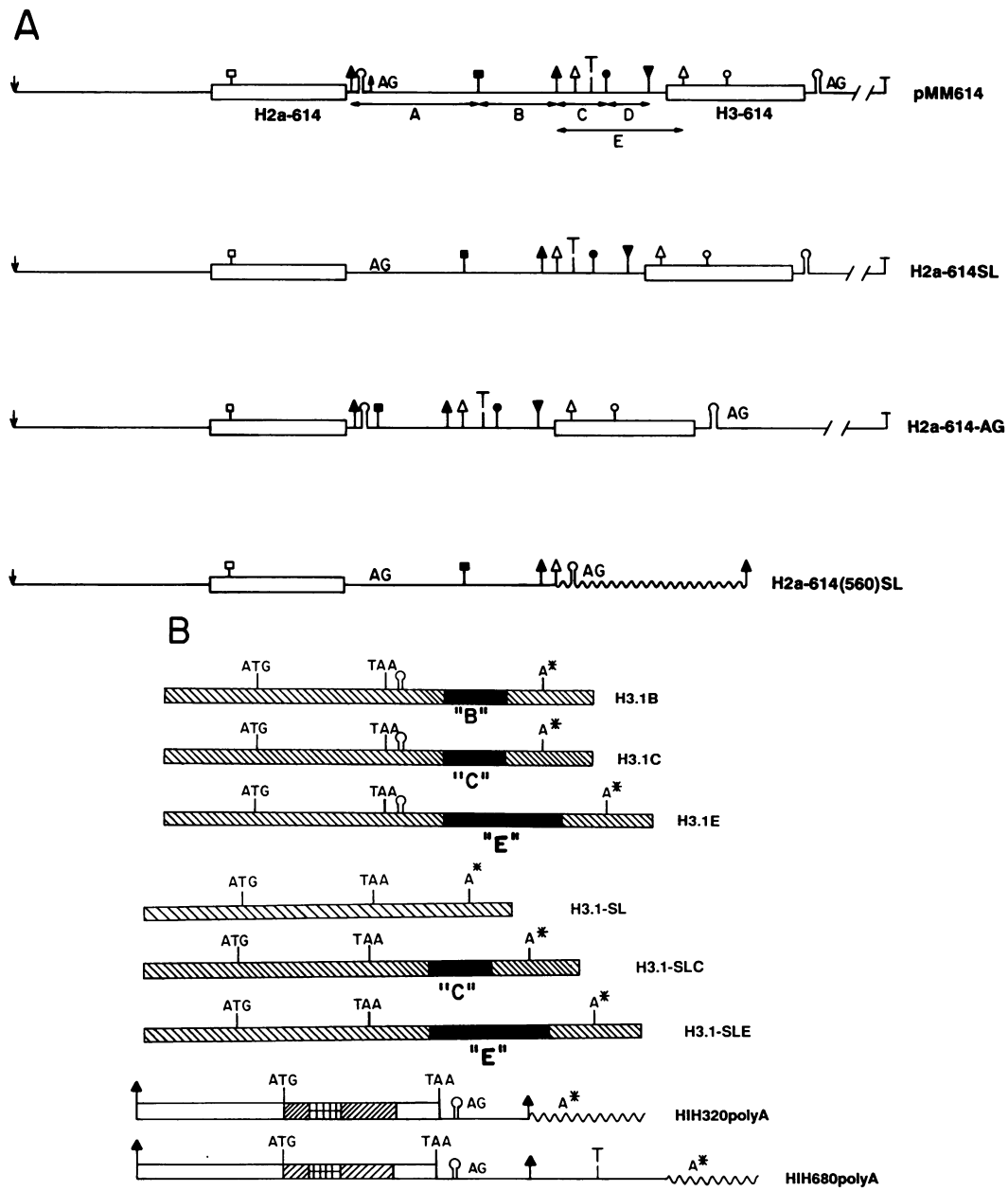


deletions of the histone 3′-end signals were constructed. The gene H2a-614-SL has a 65-nt deletion starting in the 3′ untranslated region and extending to the *Hin*fI site between the stem-loop and the U7 snRNP binding site of the H2a gene. The complete U7 snRNP binding site is retained in this gene. The gene H2a-614-AG has a 313-nt deletion from the *Hin*fI site to the *Xba*I site and contains the stem-loop but lacks the U7 binding site. Both of these constructs contain the intact H3-614 gene downstream from the modified H2a genes. The 560-nt *Sst*I-*Sst*I fragment containing the signal for 3′-end formation from the histone H2a-614 gene (10) was attached at the *Pvu*II site 560 nt 3′ of the end of the histone H2a-614 mRNA to give the gene H2a-614(560)SL. These modified H2a-614 genes are shown in Fig. 1A.

To test for termination activity, the B, C, and E fragments indicated in Fig. 1A from the intergenic region between the H2a and H3 genes were inserted at the *Eco*RV site of the H3.1-221 gene (46). H3.1B has the 200-nt *Xba*I-*Sst*I fragment inserted. H3.1C has the 140-nt *Sst*I to *Apa*I inserted. H3.1E has the 340-nt *Pvu*II fragment extending from 280 nt 5′ of the H3-614 gene to the *Pvu*II site at codon 20 inserted at the *Eco*RV site. The same fragments, C and E, were inserted into the H3.1-SL gene, which is the same H3.1 gene lacking the histone 3′-processing signal (Fig. 1B). The construction of this gene, also called the H3.21A gene, has been previously described (10).

The HIH gene, which contains the histone H3-614 promoter, part of the human α-globin-coding region including the first intron and the histone H2a-614 3′ end, has been described previously (40). The HIH320polyA gene and the HIH680polyA gene were constructed by attaching the mouse globin polyadenylation signal at the *Xba*I site 340 nt from the end of the H2a-614 mRNA or at the *Apa*I site 680 nt from the end of the H2a-614 mRNA (Fig. 1B).

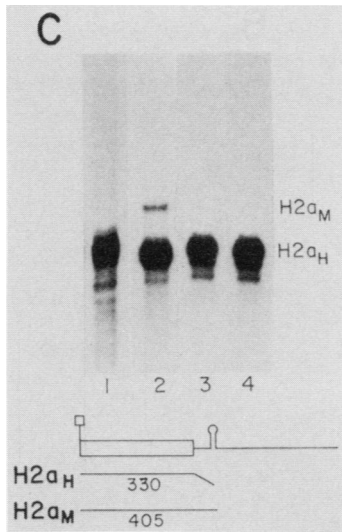


FIG. 1. (A) Deletions of the 3' end of the histone gene. The intact MM614 *Eco*RI-*Hin*dIII fragment containing the H2a-614 and H3-614 genes is shown. The H2a-614-SL gene was constructed by deleting 65 nt 5' from the *Hin*fI site, and the clone H2a-614-AG gene was constructed by deleting the *Hin*fI-*Xba*I fragment. The complete histone 3' end was attached to the H2a-614-SL gene to give the H2a-614(560)SL gene. The putative transcription termination site is indicated by a T. Fragments A through E (indicated under the intact gene) were subcloned and used as insertions into the H3.1-221 gene and cloned into bacteriophages m13mp8 and m13mp9 for use as probes for nuclear transcription assays. AG denotes the purine-rich U7 snRNP binding site required from 3' processing. The restriction enzyme site indicated are *Apa*I (●), *Hin*fI (↑), *Pvu*II (△), *Sal*I (○), *Sst*I (▲), *Sst*II (▼), *Xba*I (■), and *Xcy*I (□). (B) Insertions into the 3'-flanking region. Fragments B, C, and E shown above were inserted into the *Eco*RV site located 120 nt 3' of the end of the H3.1-221 gene to give the genes H3.1B, H3.1C, and H3.1E, respectively. The HIH gene which contains the histone H3-614 promoter, the human α-globin coding region, including the first intron and the histone H2a-614 3' end has been described previously (40). The mouse α-globin polyadenylation signal was attached at the *Xba*I site or the *Apa*I site to give the HIH320polyA or the HIH680polyA gene, respectively. A* indicates the location of the downstream polyadenylation site. Symbols: ▨, H3.1-221 gene; ■, fragment from H2a-614 gene; □, H3-614 or H2a-614 gene; ▨, α-globin-coding region; ⊞, α-globin intron; ∿∿∿, globin polyadenylation signal. (C) The intact H2a-614 gene, the H2a-614-SL gene, and the H2a-614-AG gene were each transfected into CHO cells, and the expression of the H2a-614 gene was measured by an S1 nuclease assay by using the H2a-614 gene labeled at the 3' end of the *Xcy*I site at codon 20 as a probe. A 330-nt fragment is protected by the endogenous hamster H2a mRNA ($H2a_H$) and a 405-nt fragment protected by the mouse H2a-614 mRNA ($H2a_M$). Total cell RNA (3 μg) was analyzed in each sample. Lane 1, CHO cell RNA; lane 2, RNA from CHO cells transfected with the intact H2a-614 gene; lane 3, RNA from CHO cells transfected with the H2a-614-SL gene; lane 4, RNA from CHO cells transfected with the H2a-614-AG gene.

Preparation and analysis of RNA. Histone mRNA was analyzed by an S1 nuclease assay as previously described (17). To assay the levels of mRNA from the H2a-614 gene, the gene was labeled at the *Xcy*I site at codon 20. To determine the termination site the DNA was labeled at either the 5' or 3' end of the *Xba*I site which is located 340 nt 3' to the H2a-614 gene. To analyze the mRNAs from the H3.1 genes, the appropriate construct was labeled at the 3' end of the *Sal*I sites at codon 58 of the H3.1-221 genes. S1 nuclease assays were performed as described previously (10, 17), and the fragments were analyzed on polyacrylamide gels in 7 M urea. Depending on the size of the fragment, 5 or 8% polyacrylamide gels were used.

Analysis of transcription. Isolated nuclei were prepared from mouse myeloma cells as previously described (33). The nuclei were incubated for 30 min with [α-^{32}P]CTP, and RNA was prepared as previously described. The radiolabeled RNA was divided into four aliquots (2×10^6 cpm/sample) and hybridized with single-stranded DNA for both strands of the intergenic region between H3 and H2a cloned into mp8 or mp9. Four fragments from the 3' region of the H2a-614 gene were used as probes: the 420-nt *Sst*I-*Xba*I fragment (110 C's), the 200-nt *Xba*I-*Sst*I fragment (51 C's), the 140-nt *Sst*I-*Apa*I fragment (43 C's), and the 100-nt *Apa*I-*Sst*II fragment (33 C's) which ends just 5' of the TATAA sequence in the H3-614 promoter. As an internal control, each hybridization reaction also contained single-stranded DNA for both strands of the *Sal*I-*Pst*I fragment from codons 58 to 93 of the H3-614 gene. The filters were hybridized for 72 h at 42°C in 50% formamide–0.9 M NaCl and washed as previously described (33). The filters were then treated with RNase A, washed, and exposed to X-ray film.

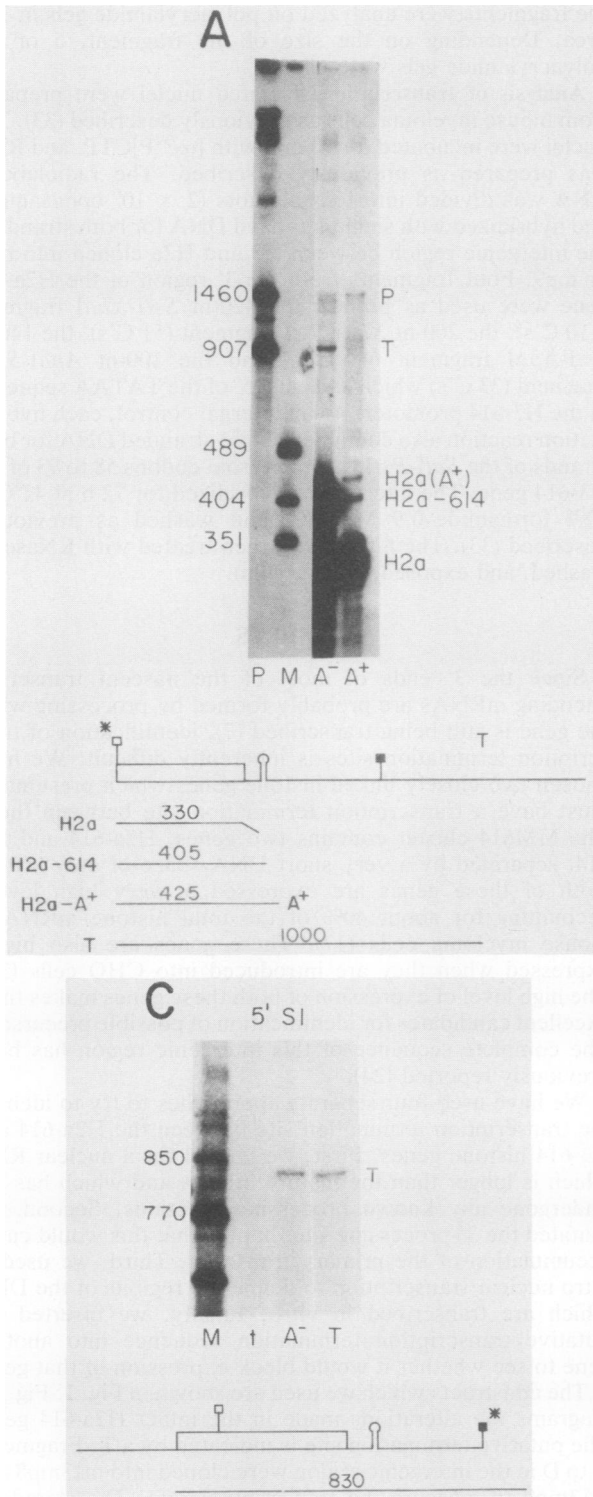
RESULTS

Since the 3' ends of most of the nascent transcripts encoding mRNAs are probably formed by processing while the gene is still being transcribed (7), identification of transcription termination sites is inherently difficult. We have chosen two closely linked histone genes which presumably must have a transcription termination site between them. The MM614 cluster contains two genes, H2a-614 and H3-614, separated by a very short DNA space of only 800 nt. Both of these genes are expressed at very high levels, accounting for about 40% of the total histone mRNA in mouse myeloma cells (17). These genes are also highly expressed when they are introduced into CHO cells (29). The high level of expression of both these genes makes them excellent candidates for identification of possible precursors. The complete sequence of this intergenic region has been previously reported (24).

We have used four separate approaches to try to identify the transcription termination site between the H2a-614 and H3-614 histone genes. First, we looked for a nuclear RNA which is longer than the mature mRNA and which has not undergone any known processing reactions. Second, we mutated the 3'-processing site, hoping that this would cause accumulation of the primary transcript. Third, we used in vitro nuclear transcription to define the regions of the DNA which are transcribed in vitro. Finally, we inserted the putative transcription termination sequence into another gene to see whether it would block expression of that gene.

The constructs which we used are shown in Fig. 1. Fig. 1A diagrams the alterations made in the intact H2a-614 gene. The putative termination site is indicated by a T. Fragments A to D in the intergenic region were cloned into m13mp8 and m13mp9 to measure the transcription rate. The stem-loop was deleted to give the gene H2a-614-SL. The purine-rich U7 snRNP binding site was deleted in the gene H2a-614-AG. In the gene H2a-614(560)SL, the stem-loop was deleted and the complete processing signal was placed 560 nt 3' of the normal histone 3' end.

Figure 1B shows the H3.1-221 genes constructed by inserting the intergenic fragments B, C, and E from the H2a-614 gene between the 3' end of the histone mRNA and the cryptic polyadenylation site 260 nt 3' to the histone



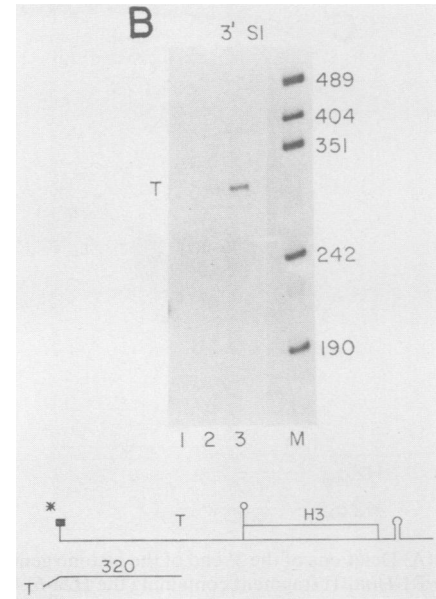
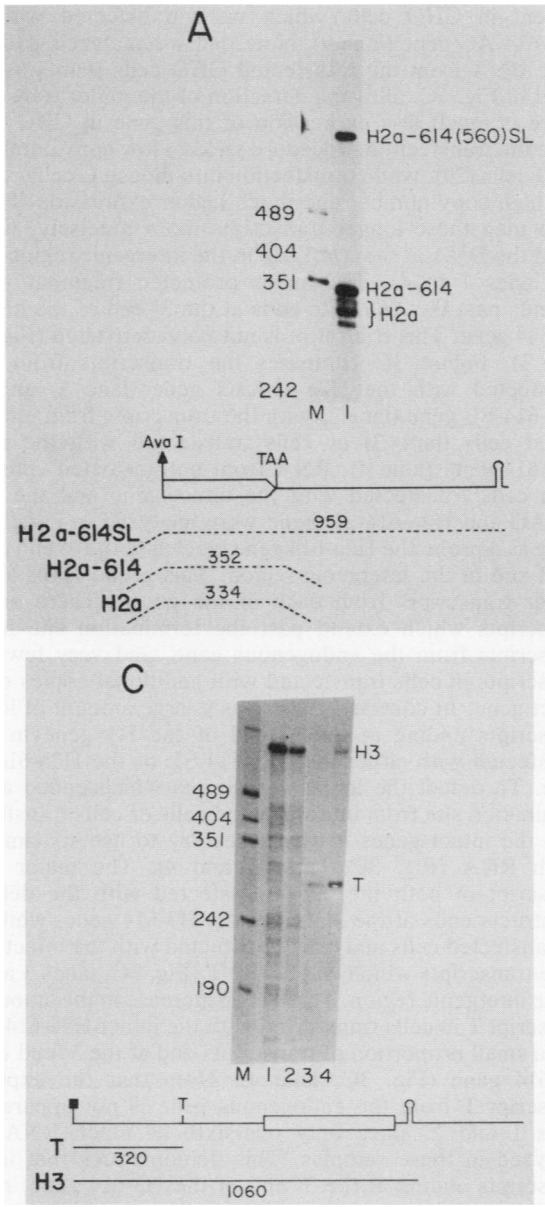


FIG. 2. Transcripts from the mouse H2a-614 gene. (A) Total cell RNA and nuclear and cytoplasmic RNA were prepared from mouse L cells. The total cell RNA was fractionated into poly(A)$^-$ and poly(A)$^+$ RNA (lanes A$^-$ and A$^+$, respectively) by chromatography on oligo(dT)-cellulose. The transcripts were analyzed by an S1 nuclease assay using the H2a-614 gene labeled at the 3′ end of the *Xcy*I site at codon 20 as a probe and then digested with *Sal*I to give a 1,430-nt probe (P). There is a 330-nt fragment protected by the mouse H2a mRNAs other than the H2a-614 mRNA (H2a) and a 405-nt fragment (H2a-614) protected by the mouse H2a-614 mRNA. In addition there were two minor protected fragments: H2a(A$^+$), 440 nt, which is polyadenylated (28, 40) and a 1,000-nt fragment (T) which extends to the putative termination site. Lane P, Labeled probe digested with *Eco*RI and *Sal*I to give 907- and 1,430-nt fragments; lane M, pUC18 digested with *Hpa*II. (B) To map the position of site T more precisely, the *Xba*I site in the intergenic region was labeled at the 3′ end and used to map cytoplasmic (lane 2) and nuclear (lane 3) RNA. Lane 1 is a tRNA control. (C) The *Xba* site was labeled at the 5′ end and used to map the 5′ end of the transcript in poly(A)$^-$ (lane A$^-$) and total cell RNA (lane T), respectively. Lane t, Analysis of tRNA. The size of marker fragments (lane M) is shown.

mRNA (10). The intergenic fragments C and E were also inserted at the same site into the H3.1-SL gene, which has the stem-loop deleted to give the genes H3.1-SLC and H3.1-SLE. Figure 1B also shows two HIH genes. The HIH gene normally gives rise to both polyadenylated and normal histone transcripts because of the presence of the intron in the gene (40). The HIH320polyA and the HIH680polyA genes have the mouse α-globin polyadenylation site attached at the *Xba*I site 320 nt 3′ of the H2a-614 histone gene or at the *Apa*I site 680 nt 3′ of the end of the H21-614 histone gene.

Both the purine-rich sequences and the stem-loop structure are required for 3′ processing of mouse H2a-614 mRNA. Two genes derived from the H2a-614 gene, one with the stem-loop region deleted (H2a-614-SL) and the other with the downstream purine-rich U7 snRNP binding site deleted (H2a-614-AG), were transfected into CHO cells. Total RNA from CHO cells transfected with the intact H2a-614 gene was analyzed by an S1 nuclease assay using the H2a-614 DNA labeled at the *Xcy*I site at codon 20. The mRNA from the transfected gene can be readily distinguished from the endogenous mRNAs (29, 30). There were two protected DNA fragments: a 330-nt fragment from the hamster H2a mRNAs (Fig. 1C, lane 1), and a 405-nt fragment from the H2a-614 mRNA (Fig. 1C, lane 2). No transcripts from the transfected genes were detected in the CHO cells transfected with either H2a-614-SL or H2a-614-AG (Fig. 1C, lanes 3 and 4). Thus, as was previously reported for sea urchins (5, 6)



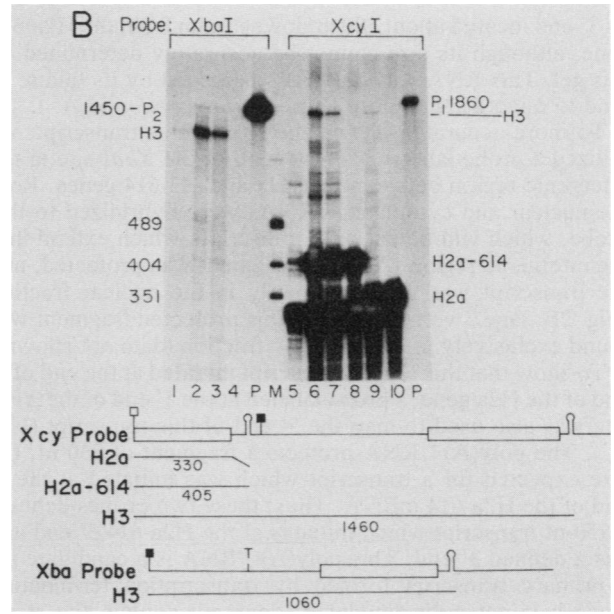


FIG. 3. Transcripts from histone genes with deleted processing signals. (A) The H2a-614(560)SL gene was transfected into mouse L cells, and the RNAs expressed from this gene were detected by S1 nuclease mapping using the H2a-614(560)SL gene labeled at the *Xcy*I site as a probe. This probe protects the endogenous H2a histone mRNAs derived from other H2a genes (H2a), the endogenous H2a-614 mRNA as a 352-nt fragment mapping to the point of the deletion (H2a-614), and the transcripts from the H2a-614(560)SL gene which end at the 3' processing site. (B) Longer transcripts from the H2a-614-AG gene. The H2a-614-AG gene with the U7 snRNP binding site deleted was introduced into mouse L cells or CHO cells, and total cell RNA was prepared. The L cell RNA was fractionated into poly(A)$^+$ and poly(A)$^-$ RNA by chromatography on oligo(dT)-cellulose. The RNAs were analyzed by S1 nuclease mapping using as a probe the H2a-614-AG gene labeled at the *Xba*I site in the intergenic region (lanes 1 through 4) or the *Xcy*I site at codon 20 (lanes 5 through 10). The *Xba*I probe was 1,450 nt, and the *Xcy*I probe was 1,860 nt. The protected fragments are H2a (protection to the TAA codon of other mouse H2a mRNAs and the hamster H2a mRNAs), H2a-614 (protection to the end of the H2a-614 mRNA), H3 (protection to the end of the H3-614 gene [1,060 nt with the *Xba*I probe and 1,460 nt with the *Xma*I probe]). Lanes 1 and 5, Poly(A)$^+$ RNA from the same amount of cells as in lanes 3 and 7; lanes 2 and 6, total RNA (5 μg); lanes 3 and 7, poly(A)$^-$ RNA (2.5 μg); lanes 4 and 8, 5 μg of total RNA from thymidine kinase-negative L cells; lane 9, RNA (20 μg) from CHO cells transfected with the H2a-614-AG gene; lane 10, RNA (20 μg) from untransfected CHO cells; lanes P, probes; lane M, pUC18 digested with *Hpa*II. (C) Transcripts from the deletion clones extend to the 3' end of the adjacent H3-614 gene. Total cell RNA from mouse L cells transfected with the H2a-614-AG gene (lane 1), the H2a-614-SL gene (lane 2), untransfected L cells (lane 3), or cells transfected with the intact H2a-614 gene (lane 4) were analyzed by S1 nuclease mapping using as a probe the H2a gene labeled at the 3' end of the *Xba*I site in the intergenic region. Lanes 1 and 2, RNA (5 μg); lanes 3 and 4, RNA (30 μg). The marker (lane M) is pUC18 digested with *Hpa*II. The fragment labeled T maps to the putative termination site and the fragment labeled H3 extends to the 3' end of the H3-614 gene.

and mice (44), both these sequence elements are necessary for 3' end formation.

Longer transcripts from the endogenous H2a-614 gene. The major RNA produced from the H2a-614 gene is a 670-nt mRNA which ends just past the stem-loop at the 3' end of the mRNA. There are two longer transcripts from the endogenous histone H2a-614 gene which are detectable in steady-state RNA in mouse L cells (Fig. 2). The S1 nuclease analysis of the 3' end of transcripts hybridized to the H2a-614 gene in total RNA from mouse L cells is shown in Fig. 2A and B. By using the DNA labeled at codon 20 in the coding region as a probe, there were several protected DNA fragments: a 330-nt fragment corresponding to the coding region of H2a mRNAs other than H2a-614 mRNA and a 405-nt fragment corresponding to the H2a-614 mRNA. In addition, there was a 440-nt fragment corresponding to a longer transcript, H2a(A$^+$), which is only 34 nt longer than the normal 3' end of the H2a-614. This transcript has been reported previously (28, 40). It is polyadenylated as judged by binding to oligo(dT)-cellulose at a cryptic polyadenylation site in the purine-rich sequence and is found in the cytoplasm (28). Another longer transcript (about 1.1 kb, labeled T) has

its 3′ end located about 600 nt downstream from the H2a-614 gene, although its size cannot be accurately determined on this gel. This RNA was poly(A)$^-$ as judged by its failure to bind to oligo(dT)-cellulose (Fig. 2A, lanes A$^+$ and A$^-$).

To more accurately define the size of this transcript, we utilized a probe labeled at the 3′ end of the *Xba*I site in the intergenic region between the H2a and H3-614 genes. Both the nuclear and cytoplasmic RNAs were hybridized to this probe, which will detect only transcripts which extend into the intergenic region. A 320-nt fragment was protected, and the transcript was found primarily in the nuclear fraction (Fig. 2B, lane 3 versus lane 2). This protected fragment was found exclusively in the poly(A)$^-$ fraction (data not shown).

To show that this longer transcript initiated at the end of 5′ end of the H2a gene, a probe labeled at the 5′ end of the *Xba*I site was also used to map the 5′ end of this transcript (Fig. 2C). The poly(A)$^-$ RNA protects a fragment of 830 nt, the size expected for a transcript which was initiated at the 5′ end of the H2a-614 mRNA. Thus, these two probes define a 1,150-nt transcript which initiates at the H2a-614 5′ end and has a defined 3′ end. This poly(A)$^-$ RNA is a candidate for a primary transcript formed by transcription termination since it is not polyadenylated, does not contain the stem-loop structure at the 3′ end, and is located in the nucleus.

Transcripts from H2a-614 genes with deleted 3′-processing signals. If the poly(A)$^-$ transcript from the endogenous H2a.2-614 gene is the primary transcript, then deletion of the processing signal might increase the amount of transcripts ending at the termination site. To test this hypothesis, the stem-loop and the U7 snRNP binding site were each deleted from the H2a-614 gene (Fig. 1A).

First we showed that if the 3′-processing signal was deleted, transcription continues 3′ of the gene. The 3′-processing signal was attached at the *Pvu*II 560 nt 3′ of the H2a-614 gene of the H2a-614SL gene, which has the stem-loop deleted, to give the H2a-614(560)SL gene (Fig. 1A). Figure 3A shows the RNAs expressed from the H2a-614(560)SL gene transfected into mouse L cells. Transcripts from this gene end at the stem-loop 560 nt 3′ of the gene, showing that transcription continues at least 560 nt 3′ of the gene. Note that the cryptic polyadenylation site which is used on a small proportion of the transcripts of the intact gene is not used efficiently when the stem-loop sequence is removed.

The other genes with deleted 3′-processing signals had the intact histone H3-614 gene 3′ of the H2a gene. Figure 3B shows the transcripts from the H2a-614-AG gene after transfection into mouse L cells (lanes 1 to 8) and CHO cells (lane 9). The transcripts were mapped with the probe labeled at the *Xcy*I site at codon 20 (lanes 5 to 10) and at the intergenic *Xba*I site (lanes 1 to 4). When the H2a-614-AG gene was transfected into mouse L cells, multiple transcripts were observed. There were two protected DNA fragments from the endogenous histone mRNAs: a 330-nt fragment corresponding to the coding region of H2a mRNAs other than H2a-614 mRNA in both the CHO and L cells and a 405-nt fragment corresponding to the endogenous H2a-614 mRNA in the mouse L cell transfection. In addition to the transcripts from the endogenous H2a genes, there were numerous larger transcripts (Fig. 3B, lanes 5 to 7), the most prominent of which was the size expected for a transcript which ends at the 3′ end of the H3-614 gene. The longer transcript was not polyadenylated (Fig. 3B, lane 7), while some of the heterogenous shorter transcripts bind to oligo(dT)-cellulose (Fig. 3B, lane 5). This longer transcript was not present in untransfected mouse L cells (lane 8) but was present in CHO cells which were transfected with the H2a-614-AG gene (lane 9). Note that we analyzed six times more RNA from the transfected CHO cells than was analyzed in Fig. 1C, allowing detection of the minor transcript. There is much less expression of this gene in CHO cells, since the transfection procedure yields a low copy number in CHO cells (29), while transfection into mouse L cells results in a high copy number and much higher expression (1).

To map these longer transcripts more precisely, we labeled the DNA at the *Xba*I site in the intergenic region (Fig. 3B, lanes 1 to 4). The major protected fragment which extends past the *Xba*I site ends at the 3′ end of the histone H3-614 gene. This transcript is not polyadenylated (Fig. 3B, lane 3). Figure 3C compares the transcripts from cells transfected with the H2a-614-AG gene (lane 1) and the H2a-614-SL gene (lane 2) with the transcripts from untransfected cells (lane 3) or cells transfected with the intact H2a-614 gene (lane 4). RNA from untransfected cells and from cells transfected with the intact gene and the H2a-614-AG and H2a-614-SL gene were analyzed in parallel by using as a probe the H2a-614 gene labeled at the 3′ end of the *Xba*I site in the intergenic region. This probe maps all the longer transcripts from each of the genes. There are no transcripts which extend past the termination site in the transcripts from the endogenous gene, and very few long transcripts in cells transfected with additional copies of the intact gene. In contrast, there was a large amount of longer transcripts ending at the 3′ end of the H3 gene in cells transfected with either the H2a-614-SL or the H2a-614-AG gene. To detect the longer transcripts which ended at the termination site from untransfected cells or cells transfected with the intact genes it was necessary to use six times as much RNA (Fig. 3C, lanes 3 and 4). The major large transcript in both the cells transfected with the deletion constructs ends at the 3′ end of the H3-614 gene, while the untransfected cells and cells transfected with the intact gene have transcripts which end at site T (Fig. 3C, lanes 3 and 4) in the intergenic region. There is an increase in the amount of transcript T in cells transfected with the intact H2a-614 gene and a small proportion of transcripts end at the 3′ end of the H3-614 gene (Fig. 3C, lane 4). Note that the expected transcript T from the endogenous gene is not apparent in lanes 1 and 2, since only one-sixth as much RNA was analyzed in these samples. This demonstrates that longer transcripts ending at the 3′ end of the H3-614 gene, rather than large amounts of transcripts ending at site T, accumulate. Thus, deletion of either portion of the processing signal results in accumulation of transcripts which extend to the end of the H3-614 gene. There is no increase in the amount of transcripts from the H2a-614-SL or H2a-614-AG genes which end at site T, suggesting that formation of this transcript requires an intact histone-processing signal. These results strongly suggest that the RNA polymerase II does not terminate until it continues past an intact 3′-end signal.

Transcription in vitro proceeds past the stem-loop and terminates upstream of the H3-614 gene. The above results suggest that transcription termination occurs in vivo about 120 nt upstream of the adjacent H3-614 gene. To assay the extent of transcription into the intergenic region, separate DNA clones for the sense and antisense strands of four regions of the histone H2a-614 gene were constructed in the M13 vectors mp8 and mp9 (Fig. 4A). Clone A starts in the 3′ untranslated region 10 nt prior to the stem-loop and extends 345 nt to the *Xba*I site. This fragment does not contain a sequence long enough to hybridize with mature histone mRNA. The other three fragments (B, C, and D) extend

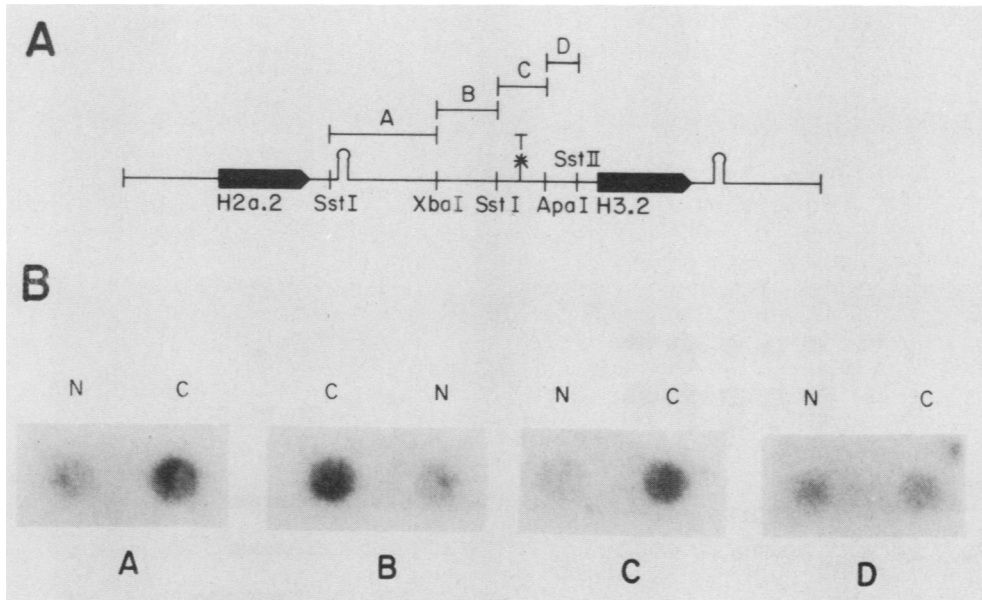


FIG. 4. Extent of transcription into the intergenic region. (A) Four subclones (A through D) extending into the intergenic region were constructed in M13 phages mp8 and mp9. (B) Single-stranded DNA corresponding to either strand of each clone was prepared and immobilized on nitrocellulose. Nuclei were isolated from mouse myeloma cells, and RNA was synthesized in vitro using [α-^{32}P]CTP as the radiolabel. The RNA was divided into four equal samples and hybridized with the two strands of each clone. The filters were washed, treated with RNase, and then exposed to the same piece of X-ray film. C denotes the transcribed strand, and N denotes the nontranscribed strand.

from the *Xba*I site to the *Sst*I site, from the *Sst*I site to the *Apa*I site, and from the *Apa*I site to *Sst*II site which is located only 20 nt upstream of the promoter of the H3-614 gene. These fragments contain 110, 51, 43, and 33 cytosines, respectively. The end of transcript T is located in fragment C. Nascent RNA transcribed from the endogenous H2a-614 gene was labeled by incubating nuclei with [α-^{32}P]CTP, and the labeled RNA was hybridized to all four cloned DNAs.

The sense strands of clones A, B, and C hybridized, while clone D did not hybridize (Fig. 4B). The amount of transcription is consistent with equimolar transcription of regions A and B and transcription partway into fragment C. The results of the extent of transcription in isolated nuclei are consistent with the end of the poly(A)$^-$ transcript T found in vivo.

The putative transcription termination sequence from the H2a-614 gene blocks the expression of longer transcripts from the histone H3.1 gene. To test the ability of the putative transcription termination site to terminate transcription in a heterologous gene is difficult because of the requirement for a functional 3'-processing signal for transcription termination. We carried out three types of experiments to demonstrate the presence of a transcription termination site. In each experiment we measured the ratio of different transcripts formed from a particular gene, a situation in which there is a built-in internal control.

The H3.1-221 histone gene produces two transcripts when it is transfected into mouse L cells, one ending at the normal histone 3' end and a longer polyadenylated transcript which ends after a polyadenylation signal located 230 nt 3' of the histone 3' end (10). To test for dependence of termination on the presence of a 3'-processing signal, we used the H3.1-SL gene (the gene also called H3.21A [10]) which lacks the 3'-processing signal. This gene gives rise to two transcripts, transcript Y ending 100 nt 3' of the normal histone 3' end and the longer polyadenylated transcript X ending 230 nt 3' of the normal histone 3' end. When this gene is introduced in multiple copies into mouse L cells, there is a large increase in the amount of histone H3 mRNA which ends at the H3.1 end (10) (Fig. 5A, compare lane 1 with lanes 2 to 5), as well as a small amount of RNA derived from the transfected gene which is polyadenylated and ends at site X. There is no polyadenylated RNA formed from the endogenous gene (10), and most (>80%) of the RNA ending at the H3.1 3' end is derived from the transfected gene. Thus, by measuring the ratio of the RNA from the transfected gene which ends at site X to the amount of RNA ending at the H3.1 3' end, we can estimate the efficiency of usage of the downstream polyadenylation site. If a transcription termination site is introduced prior to site X, we expect to see a decrease in the amount of RNA ending at site X.

To test for termination activity we inserted intergenic fragments into the *Eco*RV site 130 nt from the end of the histone mRNA and 130 nt before the the cryptic polyadenylation site in the 3'-flanking region of H3.1-221 gene. Fragment C (140 nt) which contains the putative termination site and fragment B (200 nt) which is directly 5' of fragment C were both inserted into the 3'-flanking region of the H3.1 gene to give the H3.1B and H3.1C genes, respectively (Fig. 1B). In addition fragment E (340 nt) extending from 50 nt 5' of the proposed termination site to codon 20 of the H3-614 gene was inserted in the same place to give the H3.1E gene. Note that fragment E contains the complete H3-614 promoter.

We expected less of the polyadenylated RNA, X, to be produced when the putative termination site was inserted in the 3'-flanking region of the H3.1 gene. Each of the clones was labeled at the 3' end of the *Sal*I site at codon 58 in the H3.1 gene, and the RNA isolated from mouse L cells transfected with the appropriate gene (Fig. 5A) was analyzed with an S1 nuclease assay. The 240-nt fragment results from protection of the H3-coding region of H3 mRNAs other than H3.1, and the 280-nt fragment results from protection of the

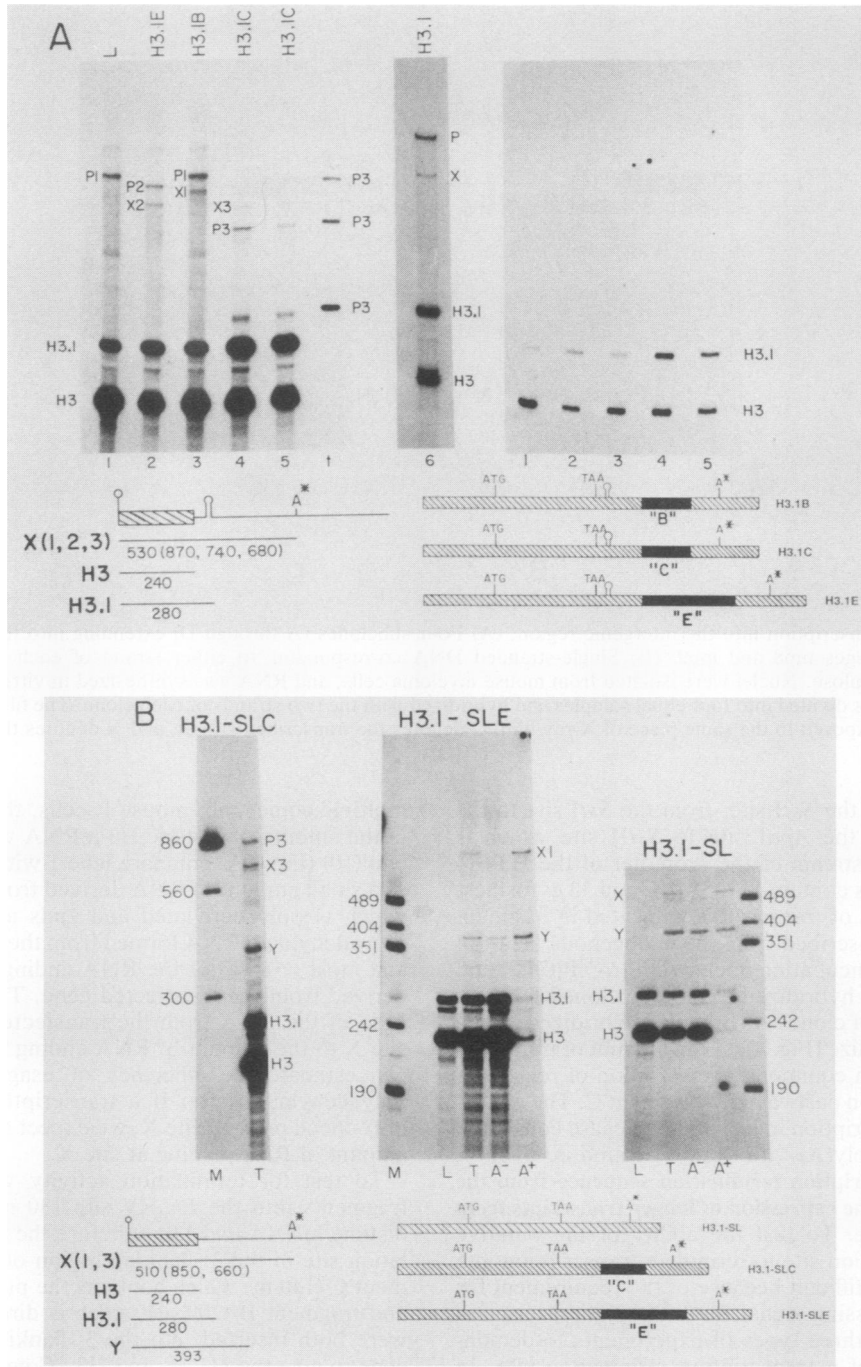


FIG. 5. The termination site blocks expression of polyadenylated mRNAs from the H3.1-221 gene and the HIH-polyA genes. (A) Total cell RNA (30 μg) from cells transfected with the H3.1B, H3.1C, and H3.1E or the intact H3.1-221 gene was assayed by S1 nuclease mapping using the appropriate gene labeled at the *Sal*I site as a probe. The genes are shown in Fig. 1B and at the bottom of panel A. The fragment labeled X, X1, X2, or X3 results from protection of the probe by transcripts which extend to the downstream polyadenylation site. This is 870 nt in the H3.1E gene, 700 nt in the H3.1B gene, 680 nt in the H3.1C gene, and 530 nt in the H3.1-221 gene. Lane 1, Untransfected L cells assayed with the H3.1E probe; lane 2, H3.1B; lane 3, H3.1E; lanes 4 and 5, duplicate cultures transfected with the H3.1C gene; lane 6, 30 μg of total RNA from cells transfected with the H3.1-221 gene analyzed by S1 nuclease mapping using the H3.1-221 gene labeled at the 3′ end of the *Sal*I site as a probe; lane t, tRNA mapped by using the H3.1C probe. The three fragments are derived from the H3.1C probe (which was a partial *Sal*I digestion due to the introduction of a *Sal*I site during the cloning procedure). The four probe bands, P, P1, P2, and P3, were 720, 1,080, 940, and 880 nt, respectively. There are two smaller bands labeled P3 (300 and 580 nt, respectively) which result from a partial digest which cleaved at a *Sal*I site introduced during the cloning procedure. The right panel is the same gel (lanes 1 to 5) exposed for 1/20 the time to show the relative amounts of H3.1 transcripts in the different lanes. Note that the large amounts in the proportion of H3.1 transcripts to H3 transcripts in lanes 2 to 5 are a result of expression from the transfected gene. (B) Total cell RNA (10 μg) from the mouse L cells transfected with either the H3.1-SLC (left panel), the H3.1-SLE (center panel), or the H3.1-SL (right panel) genes was analyzed by S1 nuclease mapping using the appropriate gene labeled at the 3′ end of the *Sal*I site as a probe (lanes T). The RNAs from the H3.1-SLE and

complete H3.1 mRNA. The largest fragment, X (which varies in length because of the different lengths of the inserted sequences), corresponds to protection of transcripts which extend to the downstream polyadenylation site.

The H3.1C gene, with fragment C inserted, expresses much less RNA X relative to H3.1 mRNA ending at the histone H3.1 3' end than the H3.1 gene, the H3.1B gene, or the H3.1E gene (Fig. 5A, compare lanes 4 and 5 with lanes 2, 3, and 6). The right panel of Fig. 5A is a lighter exposure of the same gel, showing the relative amounts of H3.1 histone mRNA in each sample. The amount of RNA ending at site X was reduced 80% by inserting fragment C compared with the other fragments. These results strongly support the interpretation that the *Sst*I-*Apa*I fragment blocks expression of transcript X, because it contains a transcription termination site. Neither fragment B nor E reduced the proportion of transcripts ending at site X. Termination is not simply due to the presence of the H3 promoter or to the length of the transcript, since insertion of the longest fragment E, which contains the complete H3-614 promoter, did not affect utilization of the 3' polyadenylation site (Fig. 5A, lane 3).

To demonstrate that the effect of fragment C in blocking transcription past the downstream polyadenylation site required the presence of the histone 3'-processing signal, fragments C and E were inserted into the H3.1-SL gene which has the 3'-processing site deleted. Figure 5B shows the results with the H3.1-SL, H3.1-SLC, and H3.1-SLE genes after transfection of the genes into thymidine kinase-negative L cells. Two transcripts are formed from these genes. Transcript Y ends 100 nt past the end of the normal histone mRNA, prior to the site where the fragments C and E were inserted, and transcript X ends at the downstream polyadenylation site described above. The mechanism of the formation of transcript Y is not known, but transcript Y is more abundant than transcript X (10). The intergenic fragments were inserted into the *Eco*RV site which lies between site X and site Y. If a functional transcription termination site is placed between these two signals, then the ratio of transcripts ending at site Y and site X should be reduced. Thus, the effect of the introduced fragments can be determined by comparing the ratio of transcripts which end at site X and site Y.

The results with the H3.1-SL gene indicate that transcript Y is two to three times more abundant than transcript X (Fig. 5B, right panel) (10). When either the 340-nt fragment E (Fig. 5B, center panel) or the 150-nt fragment C (Fig. 5B, left panel) was inserted at the *Eco*RV site which is 20 nt past the end of transcript Y, there was little effect on the relative amounts of transcripts X and Y formed. Both transcripts X and Y were formed in about the same ratio as in the gene which had no sequence inserted. As previously reported (10), transcript X was polyadenylated and transcript Y was present in both the poly(A)$^-$ and poly(A)$^+$ fractions (Fig. 5B, lanes A$^+$ and A$^-$). Thus, insertion of fragment C containing the termination site did not block formation of transcript X if there was no histone-processing signal present in the gene. This strongly supports the idea that fragment C contains a termination site which functions only after an intact histone-processing signal.

Terminator sequences prevent utilization of the globin poly(A) site placed downstream of the H2a-614 stem-loop. A second set of constructs was used to demonstrate that the termination sequence prevents the usage of the mouse α-globin polyadenylation site 3' of the histone H2a-614 gene. The polyadenylation signal from the mouse α-globin gene was attached at various distances 3' to the stem-loop. Since the polyadenylation site is not used when it is attached 3' to the intact H2a-614 gene (30, 40), the globin poly(A) site was inserted 3' of the HIH gene. The HIH gene contains the mouse histone H3-614 promoter, a portion of the α-globin-coding sequence including the first intron and the histone H2a-614 3' end. The presence of the intron interferes with normal histone 3'-end formation, resulting in the utilization of downstream polyadenylation sites (40). The major polyadenylation site utilized is a cryptic site located 34 nt 3' of the histone 3' end (40). However, if the α-globin polyadenylation site is placed 3' of the gene, the globin polyadenylation site is used and the cryptic polyadenylation site is suppressed (Fig. 6, compare lanes 1 and 2) (40). If there were a transcription termination site prior to the globin polyadenylation site, then we expect the globin polyadenylation site would not be used but, rather, polyadenylation would occur instead at the cryptic polyadenylation site which is part of the normal U7 snRNP binding site. Thus, again we can compare the ratio of transcripts from the same gene to see whether a transcription termination site is present.

The globin polyadenylation signal was inserted at the *Xba*I site 5' of the putative termination site, and at the *Apa*I site 3' of the putative termination site (Fig. 1C). When the fragment containing the globin poly(A) site was attached 5' of the termination site, 30 to 50% of the mRNAs ended at the globin poly(A) site 3' to the normal histone 3' end (Fig. 6, lanes 2 and 5) and the cryptic polyadenylation site (Fig. 6, lane 1) normally utilized in the presence of the intron is suppressed. However, when the globin polyadenylation site was placed 3' of the termination site, the polyadenylated RNAs formed ended at the cryptic polyadenylation site (Fig. 6, lane 1) and there was only a small amount of RNA produced which ended at the globin polyadenylation site (Fig. 6, lanes 1 and 4). This is consistent with the previous conclusions that transcription extends past the *Xba*I site but terminates prior to the *Apa*I site (Fig. 4). Taken together, all of these results strongly suggest that transcription terminates in fragment C and that this termination site functions only if there is an intact histone 3'-processing signal.

DISCUSSION

While much is known about the mechanism of transcription initiation, much less is known about the molecular basis of transcription termination. Histone mRNAs are unusual in that they are normally not polyadenylated. They are synthesized and exported to the cytoplasm very rapidly.The stem-loop at the 3' end of histone mRNA is involved in multiple aspects of the metabolism of histone mRNA (34, 41). While it is known that the histone mRNA 3' termini are formed by an endonucleolytic cleavage (15, 37), the site of termination of transcription of the histone genes is not known.

H3.1-SL genes were fractionated into poly(A)$^-$ and poly(A)$^+$ fractions by chromatography on oligo(dT)-cellulose, and equal amounts of each fraction were analyzed (lanes A$^-$ and A$^+$). Lane L, RNA (10 μg) from untransfected mouse L cells. Band Y, transcripts which end at the cryptic site Y in the 3' untranslated region; bands X, X1, and X3, polyadenylated transcripts formed from the H3.1-SL, H3.1-SLE, and H3.1-SLC genes, respectively, as described for panel A. Lane M, pUC18 digested with *Hpa*II (center and right panel) or the H3.1-SLC gene partially digested with *Sal*I (left panel). Band P3, Probe from the H3.1-SLC gene. A diagram of the genes and the S1 nuclease assay is shown below the figure.

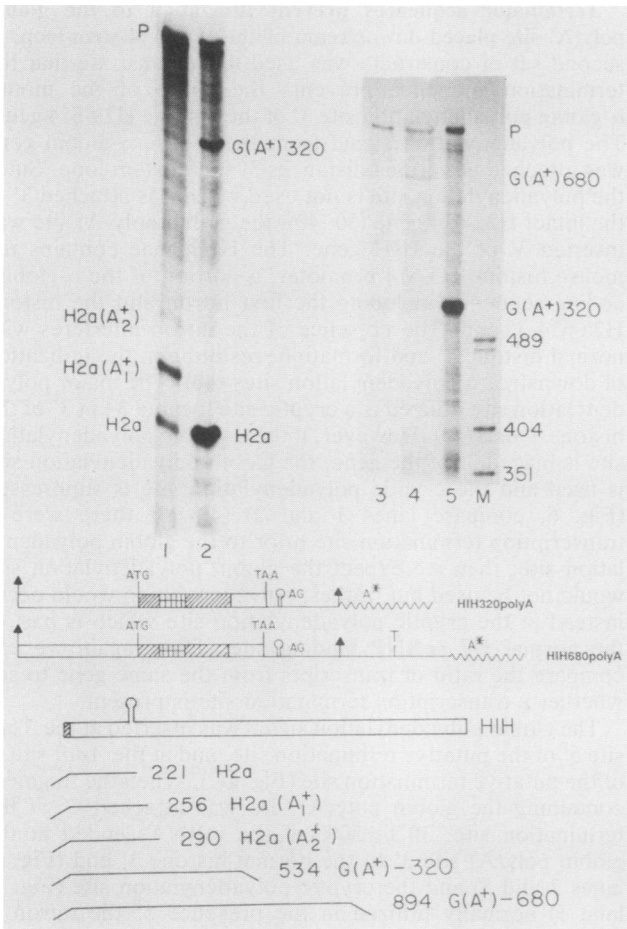


FIG. 6. Transcripts from genes containing a distal polyadenylation site. Total cell RNA (10 μg) from CHO cells transfected with the HIH320polyA or HIH680polyA genes was analyzed by S1 nuclease mapping using the HIH gene (which lacks the downstream polyadenylation sites) as a probe. The probe was labeled at the *Hin*dIII site in the globin-coding region and was 1,220 nt. The genes are shown in Fig. 1B and below the figure. Two duplicate assays are shown, and in lanes 3 through 5 the samples were electrophoresed longer to allow easy visualization of longer transcripts. The protected fragments were H2a (protection to the histone 3′ end of the H2a gene), H2a(A_1^+) and H2a(A_2^+) (protection to the cryptic polyadenylation sites utilized because of the presence of introns in the gene), G(A^+)320 and G(A^+)680 (protection to the site of attachment of the globin polyadenylation site in the respective genes). Lanes 1 and 4, RNA (10 μg) from cells transfected with the HIH680polyA gene; lanes 2 and 5, RNA (10 μg) from cells transfected with the HIH-320polyA gene; lane 3: tRNA; lane M, pUC18 digested with *Hpa*II.

We have identified a transcription termination site by three criteria and suggest that this site will only function if an intact 3′-processing signal is present. (i) We have identified a poly(A)$^-$ RNA which is present in small amounts in the cell nucleus. This RNA ends 600 nt 3′ to the end of the H2a mRNA and is initiated at the normal H2a start site. This RNA has the properties predicted for a primary transcript. (ii) By using a nuclear transcription assay we showed that there is transcription extending 3′ to the H2a gene up to the region of the termination site, but no transcripts were detected which extended further downstream. This assay placed the transcription termination site in the same region

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+467
-333
CTGCACAAACACTAGATTAGTTCTGCCCTGGGAACTTTGGGCGGCGGGCGGGGCGCGCGAGCTCCTCTT
                                                          Sst I

GGGCAGAGCCGGGACCCGCCCTCTACGGGCAGCTGCCAGACGTGGCTCCACCCTCGCAGAACGTTGGCAA
                                Pvu II
*****
GTGGACCGGGCGCGGGGCTGGACGTGGGGGGCGGGTGGGGGCGGGTCAGGTGGCGTGGCGGGCCCGAGCC
                                                          Apa I

AATGGGCGAGGTCGGGGCCGGTGACGCCACGGCCAATGGCGCGGCAGCGCGGGAGTTTCAAGTCGCTGTC

                                                          +1
TCCGCCCCGCCCCGGGGAAGACTGCGCCTATAAAGGCGGCCGGCTCGGGCCGGTATCA
    Sst II
```

FIG. 7. Sequence around the putative termination site. The sequence starting 467 nt from the end of the H2a-614 mRNA (333 nt 5′ of the start of transcription from the H3-614 gene) containing the putative termination site is shown. The *Sst*I, *Pvu*II, and *Apa*I sites are indicated. *, Region to which the end of transcript T maps. The putative Sp1 and CCAAT boxes in the H3-614 promoter are underlined. This amount of 5′-flanking region is necessary for maximal expression of the H3-614 gene (23a).

in which the poly(A)$^-$ transcript ended. (iii) To prove that this sequence actually may function like a termination site, we inserted this DNA fragment into another histone gene. A 150-nt fragment containing the termination site reduced the amount of transcripts which extended to a polyadenylation site 3′ of the normal histone 3′ end, and the activity of this fragment depended on the presence of an intact histone 3′-processing signal.

Properties of the terminator sequence. The functional termination site has been localized to a 150-nt *Sst*I-*Apa*I fragment which starts 530 nt from the end of the H2a gene and ends 100 nt 5′ to the TATAA box of the H3-614 gene. The sequence of the termination region up to the start site of the H3-614 gene is shown in Fig. 7. The end of the poly(A)$^-$ transcript is 160 nt from the TATAA site in the H3-614 gene. The transcript ends at the start of an extremely GC-rich region (87% GC for 60 nt 3′ to the end of the transcript). The region immediately upstream of the end of the transcript is 67% GC, although it is only 50% GC in the 20 nt immediately 5′ of the termination site, the lowest GC content in the intergenic region. There is no similarity to the intrinsic termination sequences defined for RNA polymerase II (26) or to the sequences in the 3′ region of snRNA genes which may be termination sites for RNA polymerase II (23, 38, 39) or to termination sites for the genes transcribed by RNA polymerase III or the mouse rRNA genes transcribed by RNA polymerase I (2, 19).

The putative termination site is in the center of the sequences required for high-level expression of the H3-614 gene (29). Deletion of the 5′ sequences to the *Sst*I site reduces expression of the H3-614 gene 70%, while deletion to the *Apa*I site reduces expression 85% (23a). Continued transcription through the termination site, which occurred when the normal processing signals were deleted, resulted in accumulation of transcripts which extended from start of the H2a-614 gene to the 3′ end of the H3-614 gene. There is not a requirement for the upstream H2a gene for complete expression of the H3 gene (29). Since the putative termination signal is in the midst of the sequences required for transcription of the H3 gene, we were not able to determine unambiguously whether deletion of the termination site from the intact gene pair affects transcription of the H3-614 gene. However, Proudfoot (42) has reported that transcripts which read through the termination in an α-globin gene interfere with expression of the downstream gene.

Coupling of processing and transcription termination. The results presented here strongly suggest that the termination

of transcription depends on the presence of an intact processing site in the histone gene. Both of the two known sequences required for proper 3'-end formation must be present. In the absence of an intact 3'-processing site, transcription continued into the next gene, with a stable RNA formed which ended at the 3' end of the H3-614 gene. In addition there were a number of different RNAs formed which bound to oligo(dT)-cellulose (Fig. 3A), suggesting that they had terminal adenosines added, even though there are no polyadenylation signals in the 3'-flanking region. Since the sequences 3' to the H2a gene are devoid of polyadenylation signals, except for the defective signal which gives rise to the small amount of polyadenylated H2a mRNA from the intact gene (28, 40), the mechanism of formation of these transcripts is not known.

There is no requirement for the presence of the termination site for efficient processing. We have previously shown that truncation of the gene which removes the termination site does not affect the amount of histone H2a-614 mRNA produced (29, 30). Simply removing the termination site by deletion also did not affect the amount of histone H2a-614 mRNA produced, although the amount of RNA produced from the H3-614 gene was reduced, at least partly because of the removal of some regions of the H3-614 promoter (unpublished data). It is likely that a major function of this termination site is to prevent readthrough into the downstream H3-164 gene, as has been demonstrated for the upstream terminator in the rRNA genes (21).

These data are consistent with the proposal that transcription terminates only after a functional poly(A) site in other genes transcribed by RNA polymerase II. Logan and coworkers (31) have demonstrated that the mouse β-globin gene termination site requires not only the region of 3'-flanking region where termination is observed to occur but also sequences including the major poly(A) addition site. Similarly, Whitelaw and Proudfoot (47) demonstrated the requirement for a functional poly(A) site for α-2-globin gene transcription termination. These results have led to the proposal that a 5' to 3' exonuclease may degrade the RNA after cleavage at the processing site and participate in the termination process (43). Alternatively the polymerase could be altered as it passes a functional processing signal (whether or not that processing signal is used) resulting in termination at a site downstream.

Connelly and Manley (13) have shown that termination on a circular adenovirus-simian virus 40 hybrid gene requires an inverted CCAAT sequence just 5' of the start of the gene. Termination occurs at this site only if there is a functional polyadenylation signal in the transcript (12). A short fragment containing this sequence is sufficient to induce termination in this gene and there is a protein which interacts specifically with this sequence which may well be involved in termination (14). There is a CCAAT sequence near our termination site, but it is not part of the fragment which confers termination on a heterologous gene. However, similar to the results of Connelly and Manley (13, 14), the complete promoter of the downstream gene is not necessary to induce termination. It is possible that factors or sequences involved in transcription termination may also be involved in transcription initiation from the adjacent gene, either directly or by preventing the transcribing polymerase from displacing transcription factors associated with the downstream gene. In the rRNA genes transcribed by RNA polymerase I, the transcription termination site near the promoter is necessary for efficient expression of the rRNA genes (18, 21, 35).

There are two possible mechanisms to explain the coupling of the 3'-end signal to transcription termination. First, there could be a termination factor which associates with the DNA at the processing site and then associates with or modifies the polymerase as it passes the 3'-processing site. Alternatively there could be an antitermination factor associated with the polymerase when it initiates transcription which is removed or inactivated as it passes the 3'-processing site. In both cases, the polymerase is prepared for transcription termination by passing an intact processing site. It seems unlikely that processing actually must occur before transcription termination takes place, since we detect complete unprocessed transcripts which presumably have terminated transcription. Our results with multiple 3' ends placed 3' of a single histone gene suggest that transcription may proceed for a relatively long distance after passing a processing site prior to processing (10, 30).

In the case of histone mRNA, there is extensive evidence that processing is regulated during the cell cycle (16, 32, 45) and that different histone genes exhibit different processing efficiencies (30). Thus, under many conditions the histone mRNA 3' end is never formed. The unprocessed transcripts do not accumulate to a significant extent (32), presumably because they are rapidly degraded. While the mechanism of destruction of these transcripts is not understood, it is likely that transcription terminates on these transcription units whether or not processing has occurred.

RNA polymerase II can clearly terminate transcription by other mechanisms other than first processing a nascent transcript. There is compelling evidence for control at the level of premature termination of transcription in a number of oncogenes (see reference 3 for examples). In the case of snRNA genes, the formation of the 3' end is tightly coupled to the promoter (22, 23, 38, 39), suggesting that transcription may terminate at a specific sequence which is recognized by factors associated with the polymerase. Thus, there certainly are other mechanisms for RNA polymerase II to terminate transcription which do not require functional 3'-processing signals.

Our results are not in agreement with the conclusions on transcription termination in the sea urchin histone H2a gene. Birchmeier and co-workers studied transcription termination of the sea urchin H2a gene in *Xenopus* oocytes (6), and Johnson and co-workers (25) studied transcription termination of the same gene in the human HeLa cell transient expression system. In both systems the termination site does not require a histone 3'-processing signal. The termination site which they defined is capable of blocking expression of a human gene in the absence of the histone 3'-processing signal, suggesting that this is an intrinsic termination signal (8). The physiological significance of this sequence is not clear, given the heterologous nature of these experiments. However, the sea urchin histone H2a termination site clearly functions differently than the site we have described here.

It is not known whether the termination signal described here is unique to histone genes or whether it might also function when coupled with other genes transcribed by RNA polymerase II.

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