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Direct demonstration of termination signals for RNA polymerase II from the sea urchin H2A histone gene

D.Briggs, D.Jackson, E.Whitelaw and N.J.Proudfoot

Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK

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ABSTRACT

Previous studies [1,2] suggested but did not prove that the sea urchin H2A histone gene possesses strong transcriptional termination signals close to, but separate from, the 3′ processing signals. In this study we have demonstrated by two independent approaches that these sequences elicit authentic transcriptional termination. First we show by nuclear run off analysis that nascent transcription terminates in the immediate 3′ flanking region of the H2A gene, in an A-rich region. Second we show that these termination signals prevent transcriptional read through when placed in the intron of a globin gene. The intronic position of the termination signal rules out any effect on steady state mRNA levels. We have therefore defined DNA sequences which act as a transcription terminator when placed in heterologous RNA polymerase II genes.

INTRODUCTION

At least three different classes of genes are transcribed by RNA polymerase II (polII): genes for polyadenylated mRNA, histone genes and small nuclear RNA genes [3]. The predominant class is poly(A)+ and is distinguished by the possession of introns as well as polyadenylation signals. Histone genes (with a few exceptions) do not possess introns and are poly(A)− [4]. SnRNA genes are similarly intronless but differ from histone genes in the signals they possess to elicit 3′ end formation [5,6]. As might be predicted from the above, the mechanism of transcriptional termination may also differ among these three different types of polII gene. Poly(A)+ genes appear to terminate transcription heterogeneously over sometimes several kilobases of 3′ flanking sequence [see 7 for review]. Interestingly this apparently random termination process requires the activity of a polyadenylation site [8,9]. SnRNA genes appear to possess a more defined termination process which occurs either at or close to the mature SnRNA 3′ terminus [10]. Authentic SnRNA 3′ end formation requires a homologous SnRNA promoter and is abolished when an heterologous polII promoter is used [11,12]. Finally histone gene termination appears to differ significantly from termination in the two other types of polII gene. In the few cases studied, termination occurs well beyond the mRNA 3′ end [1,13]. However, unlike poly(A)+ and SnRNA genes, the signals required for RNA 3′ end formation may not be required for termination: both Birchmeier et al [1] and Johnson et al [2] have studied the sea urchin H2A histone gene and show that in the absence of 3′ end processing, transcripts still fail to read through to downstream gene regions. Indeed Johnson et al [2] were able to further show that the sequence elements responsible for this phenomenon were tripartite, one part in the H2A coding sequence, an A-rich region in the immediate

3′ flanking region, and a third, more distant, 3′ flanking region. The 3′ processing signals were not required.

We describe here a continuation of the previous analysis of the H2A histone gene termination process [2]. In particular we demonstrate by nuclear run off analysis of globin-H2A histone hybrid genes transfected into human HeLa cells that authentic termination of transcription occurs in an A-rich sequence in the 3′ flanking region of the H2A gene. Furthermore we demonstrate that these termination signals function with equal efficiency whether they are placed within an intron or exon of a globin gene. We have therefore shown that unlike SnRNA genes, histone termination signals operate on transcription from heterologous polII promoters.

MATERIALS AND METHODS

1. DNA constructions

A. M13 subclones The 7 clones αF, 5′α, H2A1−4 and 3′α were constructed by purifying the fragments indicated in figure 1 from the plasmid α/H2AB pSVod [2]. These were ligated into double strand M13 MP19 linearised in the polylinker sequence at the SmaI site [14]. Clones containing both orientations of insert were isolated. A 1.6 Kb PstI fragment containing the whole human α1 globin gene was also inserted into M13MP19 in both orientations. Single strand M13 phage preparations were grown up to give sense and antisense probes for each fragment.

B. α/H2AB and the deletion mutant, Δ2 which lacks the A rich sequence of the histone termination signals, are described by Johnson et al [2].

In fact the deletion mutant Δ2 is inaccurately described by Johnson et al [2]. The 3′ end point of this deletion should be positioned approximately 100 bp 5′ to the SspI site in the 3′ flanking region of the H2A gene. Clone Δ2 therefore contains about half of the sequence present in M13 H2A3 but none of the sequence present in M13 H2A2.

C. Rabbit β-globin gene constructs The plasmid RβSVpBR328 was originally described by Grosveld et al [15]. This plasmid contains the whole rabbit β globin gene within a BglII fragment. There is 425 bp of 5′ and 355 bp of 3′ flanking sequence. A 322 bp PvuII fragment from the plasmid pUC119 containing the polylinker sequence was inserted into the ScaI site in intron 2 (Ic) and BglII site in exon 3 (Ec) of the β globin gene. Both IC and EC plasmids were linearized with SmaI in the polylinker sequence and the active sea urchin H2A termination region of 900 bp [2] was inserted in both orientations to generate plasmids I+, I−, E+ and E−. The plasmid α1 pSVed containing the human α1 globin gene was used as a cotransfection control in figure 2B [16].

2. Nuclear run-off analysis

A. Agarose bead encapsulated nuclei HeLa cells (75% confluent) were transfected with 10 μg of α/H2AB pSVod, 2.5 μg RβSVpBR328 (which provides a source of T antigen to allow replication of the SV40 origin containing plasmids) and 10 μg of HaeIII digested carrier HeLa cell DNA per 75 cm^2 flasks using calcium phosphate precipitation. Cells were then split the next day 1 in 3, and harvested 2 days later, by which time transfected cells contained an average of 100−200 plasmid copies.

Cells were encapsulated in agarose microbeads [17] at a concentration of 2.5×10^7/ml, lysed in 130 mM KCl, 10 mM Na_2HPO_4, 1 mM $MgCl_2$, 1 mM Na_2ATP, 1 mM dithiothreitol (with a final pH of 7.5) containing 0.5% Triton X100 for 30 mins on ice, and washed 5 times in buffer without Triton. The transcription reaction (1 ml final volume) contained 10^7 encapsulated nuclei in above buffer with 500 μM ATP, CTP, GTP and 100

μCi [^{32}P]-UTP at ~3000 Ci/mmol. After 15 min, 250 μM unlabelled UTP was added for 2.5 mins and the reaction was quenched by washing 2× in 10 vol of ice cold buffer. Encapsulated nuclei containing $5-10\times10^6$ cpm of incorporated [^{32}P]-UTP were made to 200 mM NaOH and incubated on ice for 10 min. The NaOH was neutralized and 2 vol of 10 mM Tris-HCl pH 7.5 1 mM EDTA, 50 mM NaCl, 1% SDS and 100 μg of tRNA added. The mixture was incubated at 20°C for 15 min and the beads removed by centrifugation at 25,000 rpm for 20 min in an SW 50.1 rotor. The RNA containing supernatant was extracted with phenol and chloroform, the RNA precipitated with ethanol and hybridized to nylon bound M13 DNA with plasmid inserts (see below). RNA prepared in this way was predominantly 100−250 nucleotides in length and contained 85−95% of the incorporated label with no contaminating DNA.

B. Standard nuclei purification

HeLa cells were transfected by calcium phosphate precipitation with 20 μg α/H2AB pSVed and 5 μg of RβSVpBR328 per 90 cM petri dish of subconfluent cells. After 2 days growth, cells were harvested (1 90 cm dish per experiment), washed in PBS and lysed by Triton (10 mM Tris-Cl pH 8.0, 10 mM NaCl, 2.5 mM $MgCl_2$, 1 mM EDTA and 0.5% NP40). Nuclei were washed in the same buffer less NP40 and resuspended in an equal volume of transcription buffer (about 100 μl) (5 mM DTT, 180 mM KCl, 10 mM $MgCl_2$, 20 mM Tris-Cl, pH 7.8, 50% glycerol). 1 μl of 10 mM ATP, GTP and CTP was added followed by 5 μl α-^{32}P-UTP (400 Ci/mmol). Following a 30 min incubation at 30°C, 20 μg of DNase was added and incubated for a further 10 min. Nuclear RNA was then purified by proteinase K digestion and further DNase digestion, using standard procedures. ^{32}P Nuclear RNA was then hybridised to single stranded M13 DNA probes immobilised on nylon filters. In detail filters were prehybridised for 12 hrs in 4×SSC , 10× Denhardts solution, 200 μg/ml sonicated DNA, 0.2% SDS and 50% formamide. The hybridisation solution was supplemented with 10% dextran sulphate and denatured ^{32}P nuclear RNA was then added. Hybridisation was carried out for 1−2 days. Filters were then washed at various stringencies using SSC concentrations ranging from 0.5×SSC to 0.1×SSC at 65°C [see 18]. Filters were prepared as follows: M13 single strand DNAs (about 1 μg/0.5 cm slot) were fractionated on agarose gels and transferred by blotting to nylon filters (Amersham Hybond N). DNA was bound to the filter by UV light according to protocols provided by Amersham.

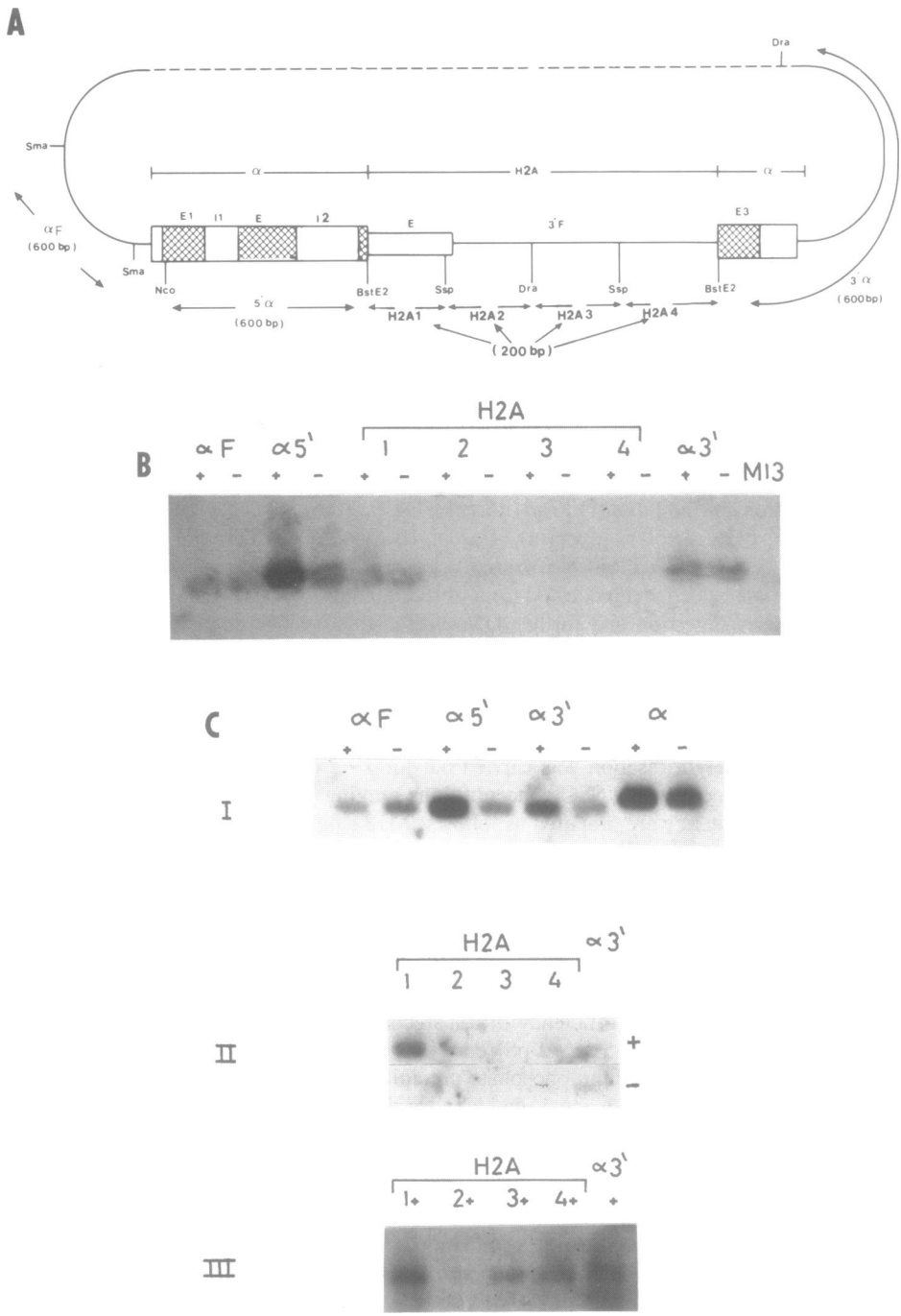
3. Analysis of steady state rabbit β globin/H2A mRNA

HeLa cells were transiently transfected with RβSVpBR328 and various derivations containing the H2A termination sequence (EC, IC, E+/−, I+/−). α1 pSVed was cotransfected to control for efficiency of transfections between different experiments. 20 μg of RβSVpBR328 and 2−5 μg α1 pSVed was precipitated onto subconfluent HeLa cells (90 cm petri dishes) by calcium phosphate precipitation. Cytoplasmic RNA was purified and subjected to S1 analysis using 3′ end labelled double strand DNA probes as described in figure 2C [see 18].

RESULTS

Nuclear run off analysis on histone H2A termination signals

The only direct approach to identify termination of transcription is to measure the density of polymerase molecules along a gene sequence. This can in principle be achieved by nuclear run off analysis which under appropriate conditions, end labels the nascent RNA transcripts [19]. Furthermore this approach has been successfully applied to map the site of termination


A
Dra
Sma
α
H2A
α
E1
I1
E
I2
E
3'F
E3
αF
(600 bp)
Sma
Nco
5'α
(600 bp)
BstE2
Ssp
Dra
Ssp
BstE2
3'α
(600bp)
H2A1
H2A2
H2A3
H2A4
(200 bp)
B
H2A
αF
α5'
1
2
3
4
α3'
+ - + - + - + - + - + - + - + - M13
C
αF
α5'
α3'
α
+ - + - + - + -
I
H2A
α3'
1 2 3 4
II
+
-
H2A
α3'
1+ 2+ 3+ 4+ +
III

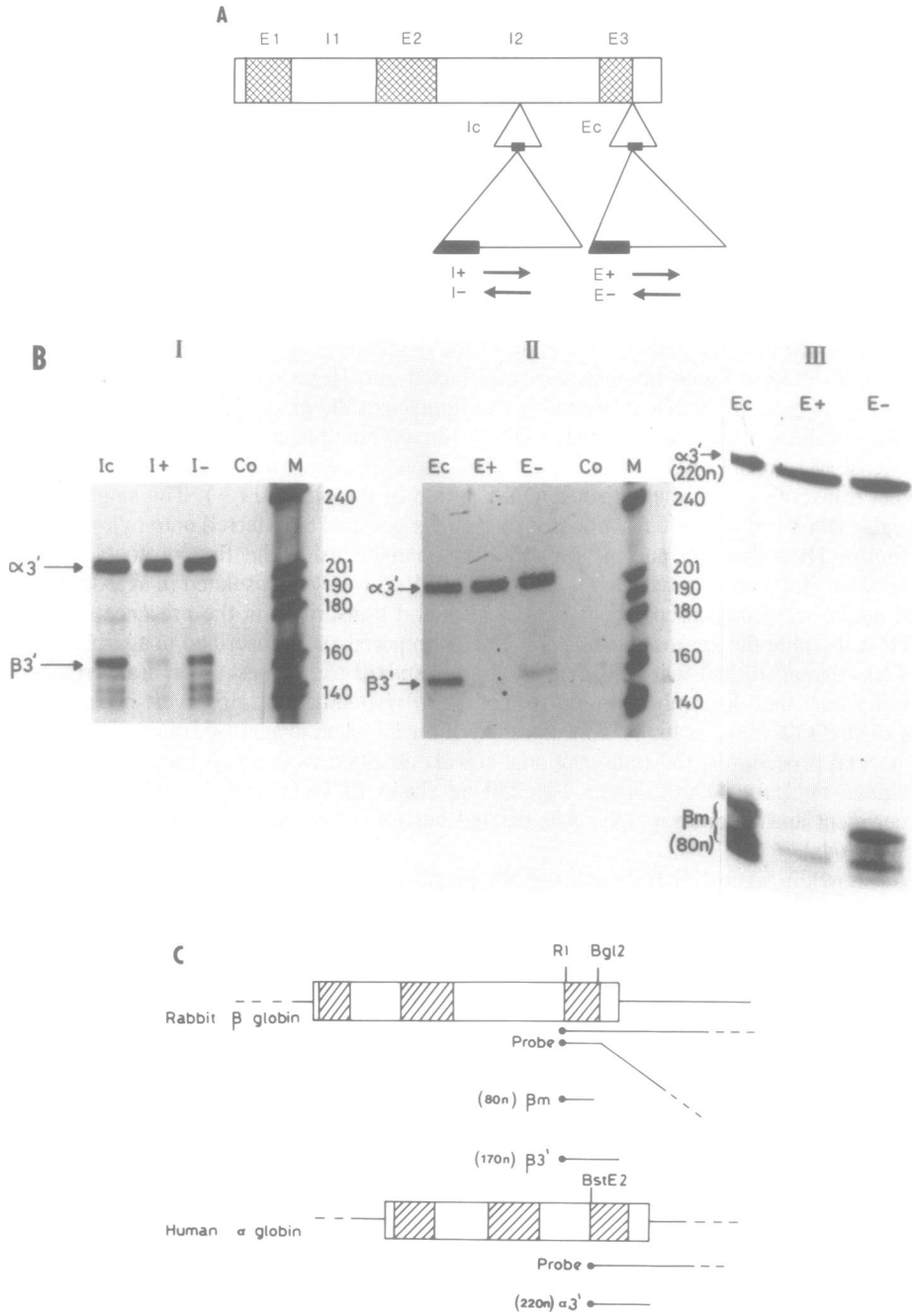
in a number of highly expressed, endogenous genes. However it is harder to obtain reliable data for termination of transcription in genes which are transiently transfected into tissue culture cells, as part of a replicating plasmid. In general non specific transcription from all parts of the plasmid may obscure an authentic termination process. We have employed two different nuclear run off techniques to study the H2A termination process in transient expression assays. Both sets of data give the same result confirming the validity of our interpretation.

Figure 1A shows a diagram of the plasmid α/H2A pSVod which we had previously constructed to study H2A termination [2]. This plasmid contains the human α1 globin gene and SV40 origin of replication sequences to allow high level expression of α globin mRNA following transfection into tissue culture cells (HeLa). The H2A termination sequences are inserted into the 3rd exon of the α globin gene. To probe for nuclear run off signals obtained with this plasmid (transfected into HeLa cells) we cloned the seven α and H2A gene fragments indicated in the figure into the phage M13 MP19 [14]. Both sense and antisense single strand M13 DNA clones were obtained for each fragment. The antisense M13 probes will detect α/H2A transcripts (+) while the sense M13 probes will detect transcription off the antisense DNA strand of the plasmid (−). The single strand circular DNA probes were fractionated on agarose gels and transferred onto nylon filters.

Figure 1B shows the nuclear run off data obtained using the first protocol. In this procedure HeLa cells transfected with α/H2A pSVod were encapsulated in agarose beads and nuclei were then prepared by Triton lysis and transcribed in the presence of α-^{32}P UTP still within the agarose beads [17]. Nuclei prepared and transcribed in agarose beads are less damaged than when purified by more standard techniques. Their transcriptional activity may therefore more closely reflect the *in vivo* situation. However, because it is impossible to achieve as high a concentration of nuclei when in agarose beads as compared to normal procedures, the transcriptional signals obtained were much lower. To achieve adequate nuclear run off signals 10×250 ml flasks of HeLa cells were used in each experiment and autoradiography was carried out for a two week period [see Materials & Methods].

As shown in figure 1B, ^{32}P nuclear RNA purified from the transfected HeLa cell nuclei transcribed in agarose beads was hybridised to filters with the three longer M13 probes (~600 nucleotides), αF, α5′ and α3′. All three probes gave significant antisense signals (−). These signals probably reflect non specific transcription off the α/H2A pSVod plasmid,

Figure 1 *A. Diagram of plasmid α/H2AB pSVod* [2].
The positions of the various fragments subcloned into M13MP19 to be used as nuclear run off probes are indicated as well as their approximate sizes. Hatched boxes denote α globin exons; stippled box denotes H2A gene sequence. Open boxes denote α globin gene non-coding and intron sequences; line denotes α-globin and H2A gene flanking sequence and vector sequences.
B. Nuclear run off analysis of α/H2AB pSVod
Data obtained using agarose bead encapsulated nuclei [17]. Figure shows autoradiograph of a filter with bound M13 DNA probes hybridised to ^{32}P nuclear RNA. The positions of the different probes are indicated. + denotes mRNA sense transcript. − denotes antisense transcript.
C. Data obtained using standard nuclear run-off procedure [19].
I.Longer α globin M13 DNA probes αF, α5′ and α3′ including an M13 DNA probe containing the whole α globin gene were used.
II and III. Shorter H2A M13 DNA probes were hybridised to nuclear RNA obtained from α/H2AB and Δ2 α/2AB respectively.


A
E1
I1
E2
I2
E3
Ic
Ec
I+
I−
E+
E−
B
I
II
III
Ic
I+
I−
Co
M
Ec
E+
E−
Co
M
Ec
E+
E−
α3′
β3′
240
201
190
180
160
140
α3′
(220n)
βm
(80n)
C
R1
Bgl2
Rabbit β globin
Probe
(80n) βm
(170n) β3′
BstE2
Human α globin
Probe
(220n) α3′

since no active promoter is present other than that for α globin. Interestingly only α5′+ gave an α/H2A transcript signal above this background level (about 10-fold). αF+ and α3′+ gave background signals. This suggests that transcription initiated on the α promoter but failed to read through the H2A sequence into the 3′ side of the α gene. Termination of transcription must therefore be occurring within the H2A sequence. To map more precisely where termination occurs in the H2A sequence, four M13 probes, H2A1, 2, 3 and 4, were used, each containing about 200 nucleotides of H2A sequences (figure 1A). As indicated, signals were obtained for both sense and antisense transcription in H2A1, which corresponds to the 3′ end of the H2A coding sequence, but no signals were obtained for the other three H2A probes. Clearly the background signal for these shorter 200 nucleotide M13 probes is much lower than for the longer 600 nucleotide M13 probes (αF, α5′ and α3′). We therefore infer that termination of transcription occurs soon after the 3′ end of the H2A histone gene. Furthermore an antisense transcript appears to initiate from the same region where H2A termination occurs.

Figure 1C shows nuclear run off data on nuclei purified from α/H2A pSVod transfected HeLa cells using more standard nuclear run off procedures. In this case nuclei were purified by Triton lysis of the HeLa cells and were resuspended in a standard transcription buffer with α-^{32}P UTP [see Materials & Methods]. As before α+5′ gives a strong signal as compared to α+3′ indicating termination in the H2A sequence [Fig.1CI]. However, slightly more transcription appears to read through to α+3′ in this experiment, although there is still a 5-fold drop in signal between α+5′ and α+3′. At least an 80% termination level is therefore obtained. Again with the H2A1−4 probes (Fig.1CII), nuclear run off signals can be detected in the 3′ end of the H2A gene but not in the 3′ flanking region (i.e. in H2A1 but not H2A2, 3 and 4). In fact we do see a faint signal in H2A2 which more closely maps the termination process to the A-rich region in the 3′ flanking region of H2A. No signals were detectible above background using the antisense M13 probes (H2A 1, 2, 3, 4)—except a faint signal for H2A1—which was seen more clearly in figure 1B as discussed above. Finally figure 1CIII shows nuclear run off analysis of a deletion mutant of α/H2A which lacks the A-rich 3′ flanking sequence. In this case nuclear run off signals are detectable in H2A1, H2A3 and H2A4. H2A2 sequence is wholly deleted, while H2A3 sequence is only partially deleted. Therefore H2A2 gives no significant signal, while H2A3

Figure 2 *A. Diagram of the rabbit β-globin gene* indicating the positions of the pUC119 polylinker inserts as well as the H2A termination sequences.
Hatched boxes denote globin exons. Open boxes denote globin non-coding and intron sequences. The filled-in box in the pUC119 insert indicates the polylinker sequence. The filled-in box in the H2A insert denotes H2A gene sequence while the line denotes 3′ flanking sequence.
B. S1 analysis of the various RβSVpBR328 plasmid transfections
S1 analysis of cytoplasmic RNA from HeLa cells transfected with plasmids Ic, I+, I−, Ec, E+ and E− RβSVpBR328 and cotransfected with α1 pSVed.
Panels I and II used the β 3′ probe shown in 4B. In fact the RNA produced by Ec, E+ and E− has extra RNA sequence at the BglII site, the site of the pUC119 polylinker insert. However the DNA S1 probe was not cleaved at this position under the S1 conditions used. Panel III confirms the data obtained in panel II using an S1 probe that completely diverges from the RNA sequence at the BglII site. All three panels include S1 analysis of α globin mRNA produced by the cotransfection control plasmid.
C. Diagrams of the rabbit β globin and human α globin genes showing the positions of the DNA probes used and the mRNA signals obtained.

does give significant signals. This result demonstrates that the H2A A-rich region is required for termination as previously suggested by Johnson et al [2].

Histone H2A termination signals function equally efficiently when placed in the intron or exon of a globin gene

As an independent and less laborious approach to study termination, we inserted the H2A termination signals into the rabbit β globin gene, transfected these Rβ/H2A constructs (which are in a transient expression plasmid vector) into HeLa cells and then measured steady state mRNA levels using S1 analysis. We have previously demonstrated that when these same H2A signals were placed in the 3rd exon of the human α globin gene, steady state transcripts were not detectable beyond the termination region. In fact, the α/H2A mRNA produced was reduced about 10 fold as compared to α globin mRNA due to the lack of an efficient poly(A) site in the H2A sequence [2]. However in this previous study we could not formally rule out the possibility that the H2A termination signals might prevent steady state mRNA accumulation by destabilizing the mRNA rather than by promoting termination. To test this possibility we have inserted the H2A termination signals both in the middle of the large intron of the rabbit β globin gene as well as in its third exon. We reasoned that any effect on mRNA levels by the H2A sequences when placed in the intron could not exert their effect on mRNA stability, since the H2A sequences would be spliced out of the mature mRNA.

Figure 2A shows a diagram of the various Rβ/H2A constructions obtained. To facilitate cloning we first inserted a fragment of the plasmid pUC119 containing its polylinker sequence into both the middle of the large (0.6 kb) intron 2 (Ic) as well as the middle of exon 3 (Ec). Both orientations of the H2A termination region were cloned into the middle of the polylinker sequence to generate constructs I+/− and E+/−.

Figure 2B shows S1 nuclease RNA mapping of cytoplasmic RNA obtained from HeLa cells transfected with these various Rβ/H2A constructs. The positions of the probe and S1 digestion products obtained are aligned with a map of the rabbit β globin gene in figure 2C. As shown in figure 2B, panel I, placing the H2A termination region in the intronic position drastically reduced the levels of β globin mRNA reading through to the 3′ end of the gene (I+). Furthermore this reduction in β 3′ signal was orientation dependent and not caused by the pUC119 polylinker fragment since both Ic and I− gave nearly equal levels of β 3′ signal. Similarly when placed in the exonic position (see E+ of panel II), the H2A termination region greatly reduced the level of β 3′ signal as compared to the Ec control with just the pUC119 polylinker but not H2A sequence. However in the reverse orientation (E−), the H2A termination signal did cause significant reduction of the β 3′ signal although to a lesser extent than in E+. Panel III confirms these results on Ec, E+ and E− using a different S1 probe which gave mismatch bands of about 80 n corresponding to β globin mRNA. As before, E+ abolished the β mRNA signal while E− has reduced but significant levels of β signal as compared to Ec. Each construct was cotransfected with a plasmid containing the human α globin gene. Using a DNA probe to detect α globin mRNA 3′ ends (Figure 2B), the level of α globin S1 signal is the same for all six transfections demonstrating that the lower level of β mRNA signal in E−, E+ and I+ was not due simply to inefficient HeLa cell transfection. These results suggest that the H2A sequence when placed in the intron (I+), functions as an orientation specific terminator since I+ gives a low signal while I− gives a normal signal. At most, only 20% of transcripts read through the H2A sequence to give low levels of β/H2A mRNA.

The fact that the H2A sequence reduces mRNA levels by over 80% in the forward

(5') $A_3TA_2TA_3TA_3TA_3TATATA_4CA_5TGA_2C_2A_3GTA_2TA_2TA_2TAGTA_3$ (3')

(3') $T_3AT_2AT_3AT_3AT_3ATATAT_4GT_5ACT_2G_2T_3CAT_2AT_2AT_2ATCAT_3$ (5')

Figure 3 *Sequence of the H2A termination A rich sequence*
The top strand sequence is in the mRNA sense. The antisense bottom strand sequence shows the positions of the multiple T_3A sequences that will be present in the Rβ/H2A constructs I− and E−. These T_3A repeats may act as mRNA destabilizers [20].

direction (E+) but also to some extent in the reverse direction (E−), when placed in the third exon, suggests that the H2A terminator sequence may have a mRNA destabilization effect in reverse orientation. As described above, termination of transcription occurs in or close to the A-rich region in the 3′ flanking sequence of the H2A gene. Indeed this A rich region is required for the termination process as shown previously [2; and in figure 1CIII). Figure 3 shows the nucleotide sequence of this A rich region. As indicated, this sequence contains multiple copies of the short repeat AAAT. This repeat sequence therefore generates several AATAAA sequences which would potentially act as polyadenylation signals. Indeed, Johnson et al [2] demonstrated that these sequences are capable of inefficient polyadenylation. Furthermore, in the reverse orientation this A_3T repeat sequence forms a UUUA repeat identical to the well established RNA destabilizer sequence of Shaw and Kamen [20]. As a mRNA destabilizer, this sequence should act at the mRNA level and have no effect when placed in the intron of a gene. Consistent with this explanation, we found that E− but not I− caused some reduction in mRNA levels although not as large a reduction as in the forward direction (E+). In summary then, the H2A sequence functions as a terminator equally well in the intron or exon of the β globin gene. However the A rich region may act as an mRNA destabilizer sequence when positioned in reverse orientation in the exon position.

DISCUSSION

The experimental results presented here demonstrate that the 3′ coding region and 3′ flanking sequence of the sea urchin H2A histone gene together elicit transcription termination when placed within heterologous globin genes. These results further reveal that actual polymerase release must occur near or at the A rich region, 200 bp into the 3′ flanking region of the H2A histone gene.

Using two different nuclear run off protocols, we show the disappearance of nuclear run off signals over the A rich region and demonstrate that when this A rich region is deleted, transcription proceeds through the rest of the H2A 3′ flanking region. Also associated with termination at the A rich region is an antisense transcript apparently initiating within the A rich region. Although we have not investigated this antisense transcript further, it seems plausible to us that the high local concentration of RNA polymerase II at the A rich termination site might promote fortuitous transcription off the opposite DNA strand.

The experiments described here also provide an alternative approach to the study of polII termination signals which may prove especially valuable in view of the difficulty often encountered in obtaining reliable nuclear run off data. Thus we have demonstrated that the H2A termination signals largely prevent steady state read through transcription when cloned into the intron of the β globin gene in the correct orientation. The possibility that the H2A sequence destabilizes mRNA can be ruled out since no H2A sequences are in the mature spliced mRNA. Indeed the problem of distinguishing between termination and mRNA stability is exemplified by the result that the A rich region of the H2A

termination signal may form an mRNA destabilizer in reverse orientation when placed in an exonic rather than intronic position.

A key question to address from the outcome of these experiments is the question of how general the polII termination process described here for the sea urchin H2A histone gene is to other histone genes and possibly all polII genes. It may well turn out that the tandem repeat structure of the sea urchin histone gene cluster with closely spaced genes places special transcriptional constraints. Efficient termination between such genes may be important to prevent transcriptional interference between adjacent genes [16,21,22].

Finally it should be noted that we have tested the sea urchin H2A terminator in an heterologous system. That is, we have placed it within mammalian globin genes expressed in human tissue culture cells. The lack of tissue culture systems for sea urchins in part forced us to use this experimental system. However it should be remembered that many aspects of transcriptional initiation in polII genes are highly conserved between yeast and mammals, for example the CCAAT box [23] and TATA box binding factors [24,25]. The possibility then that transcription mechanisms such as termination are equivalent between sea urchin and mammals seems highly plausible. Irrespective of these considerations it is clear that we have defined an efficient polII termination process which functions in mammalian cells and therefore most probably represents the physiological termination process of the sea urchin H2A histone gene.

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