Initiation and termination of human U1 RNA transcription requires the concerted action of multiple flanking elements

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ABSTRACT

Sequences in the 5' flanking region of small nuclear RNA (snRNA) genes are responsible for recognition of 3' end signals. Formation of the pre-U1 3' end occurs at the downstream signal closest to the promoter, probably by transcription termination. We have analyzed promoter elements for their participation in formation of the 3' ends of pre-U1 RNA. To do this, a human U1 RNA gene with deletions in individual promoter elements was microinjected into *Xenopus laevis* oocytes and the resulting RNAs were analyzed by a nuclease S1 protection assay. Each of the promoter elements, except element B (the functional equivalent of a TATA box), was shown to be dispensable for recognition of the snRNA 3' end signal. This latter element was necessary, but not sufficient, for initiation of transcription; so its possible role in termination could not be assessed. Therefore, it is likely that recognition of the 3' end signal is an inherent feature of transcription complexes that initiate at an snRNA promoter.

INTRODUCTION

The abundant small nuclear RNAs U1–U5 are synthesized by an RNA polymerase II that closely resembles that which is used for synthesis of mRNAs. Transcripts from snRNA genes initially have m⁷G caps, like mRNAs (1–5), and synthesis of both snRNAs and mRNAs is inhibited by α -amanitin (6–7), 5,6-dichlorobenzimidazole riboside (8), monoclonal antibodies to RNA polymerase II (9), and temperature-sensitive mutations in RNA polymerase II (10). Also, snRNA promoters contain potential binding sites for mRNA transcription factors (11–17). In spite of these similarities between snRNAs and mRNAs, significant differences in the 5' and 3' flanking regions of their genes indicate that the transcription of these two types of RNA are not equivalent (18).

At least four functionally discrete sequence elements in the 5' flanking region of the human U1 snRNA genes contribute to the accumulation of the snRNA (Figure 1), two of which are shared with other snRNA genes (reviewed in references 18 and 19). One of these is the proximal element, PSE, or element B that functions like a TATA box of an mRNA promoter by positioning the transcription initiation site. This element differs from a TATA box in that it is located 50–60 base pairs from the start site instead of 30, and its sequence is not homologous to conventional TATA boxes in mRNA promoters. The other, the distal element, DSE, or element D functions like an enhancer by stimulating transcription up to 50-fold when it is present in either orientation upstream of the initiation site (20). Although this element and similar sequences from U2 genes can bind to the same transcription factors that activate mRNA promoters (11, 15, 21), they cannot be substituted functionally for mRNA enhancer elements (13, 21–23). Two other promoter elements, A and C, each contribute about 5-fold to the efficiency of U1 synthesis (20).

Formation of the 3' end of the earliest detected snRNA precursors (pre-snRNAs) occurs, probably by termination, 1–10 nucleotides downstream of the mature 3' end (24–27) in response to a conserved sequence GTTYN_{0–3}AAARRYAGA located about 14 nucleotides further downstream (28–30). This is in contrast to transcription of pre-mRNA, which usually terminates heterogeneously far downstream of a cleavage and polyadenylation site (reviewed in references 31–33). Efficient recognition of the snRNA 3' end signal requires that transcription be from an snRNA promoter, since substitution of an mRNA promoter results in elongation of most transcripts past this signal and subsequent cleavage and polyadenylation at a downstream polyadenylation site (3, 30, 34). Consequently, less than 20 percent of the RNAs transcribed from an mRNA promoter have 3' ends made in response to the snRNA 3' end signal.

The purpose of the experiments reported in this paper was to determine which parts of the promoter for human U1 RNA are required for initiation of transcription and for recognition of the 3' end signal. We find that each one of the functionally defined 5' flanking regions, with the exception of the proximal element (B), is dispensable for both transcription initiation and 3' end signal recognition. Curiously, element B alone is not sufficient for initiation of transcription, indicating that additional 5' flanking region sequences are needed. Since element B is required for transcription, its role in recognition of the 3' end signal could not be tested directly. Thus, our data show that correct initiation and termination of snRNA synthesis cannot be uncoupled.

MATERIALS AND METHODS

DNA templates

All constructs contained part or all of the U1 promoter and coding sequence derived from the human gene HU1-1D (35) cloned in the vector pAT153, a derivative of pBR322 (36). Plasmids with a XhoI linker inserted between positions +27 and +28 (relative to the U1 cap site) to generate a marked maxi-U1 coding region, with deletions into the 3' end signal to +189 and +185, and with a wild type promoter or a deletion into element D have been described previously (30). Ligations and other manipulations of DNA were performed according to reference 37. To place the maxi-U1 gene downstream of other mutant promoters, we isolated BglII-HindIII fragments that included U1 sequences from position –6 to +189 or +185 (with or without a functional 3' end signal, respectively) and sequences from SV40 sufficient for cleavage and polyadenylation of its late mRNA (30). Each of these fragments was used to replace existing coding and downstream sequences (between the BglII and HindIII sites) in the following promoter deletions which have been described previously: –35/–8, –105/–45, –105/–67, –105/–80, –129/–105, and –138/–105 where numbers indicate the positions of the deletion endpoints (20). The sequences removed between element B and the cap site in the –35/–8 mutant were replaced by insertion of a 35 base pair Sau3A fragment from pBR322 (between base pairs 4011 and 4046) (38) in the BglII linker at position –6 opposite to its normal orientation, thus generating the –35/–8R1 plasmids (+185 and +189). To construct the plasmids with mutations in elements D, C, and A, the –35/–8R1 plasmids were digested with PvuII (at position –105) and SphI (at base pair 561 in pAT153). The ends of the DNAs were made blunt using T4 DNA polymerase and all four deoxynucleotide triphosphates before plasmids were recircularized with T4 DNA ligase. To generate U1 templates that lacked the entire promoter (drawn at the bottom of Figure 1), the –35/–8R1 plasmids were

linearized at the XhoII site at -35 and then recut at the SphI site (at position 561 in pAT153). The ends were made blunt and the plasmids recircularized as above.

To duplicate the coding region in U1+185/Bi33, U1+189/Bi33, and U1+199/Bi33, the BglII-EcoRI fragment that includes the coding region of the U1 maxi-gene Bi33 (5) from position -6 to +199 and part of pAT153 was inserted in place of the BamHI-EcoRI region of U1+185, U1+189, and U1+199 (30), respectively. To generate Bi33/U1+199, a BglII-EcoRI fragment that spans the region from position -6 to +199 of the wild type U1 gene along with part of pAT153 was inserted in place of sequences between the BamHI site (at +199) and the EcoRI site downstream of Bi33 coding region (5).

Nuclease S1 probes

Single-stranded, uniformly labeled DNA probes complementary to U1 RNA were generated by primed synthesis on single-stranded M13 templates (described below) essentially according to reference 39. In a typical 1.0ml reaction, 80 µg of single-stranded M13 DNA was mixed with 2 µg of the 17-mer M13 universal primer (5'-dGT AAAACGACGGCCAGT-3', 40) and then incubated with 350 µCi [α -³²P] dCTP (3000Ci/mmol, Amersham), 15 µM dCTP, 1mM dATP, 1mM dGTP, 1mM dTTP, 50mM NaCl, 10mM Tris-HCl (pH 7.6), 10mM MgCl₂, 1.0mM dithiothreitol, 100 µg/ml bovine serum albumin and 100 units of *E. coli* DNA polymerase Klenow fragment (Promega Corp.) at room temperature for 30 minutes followed by restriction at the PvuII site (at position -105). Probes were purified by preparative gel electrophoresis through 4.5% polyacrylamide (19:1), 7M urea, 89mM Tris-borate (pH 8.3) and 2.8mM EDTA (1×TBE) followed by elution fundamentally according to reference 41.

To generate probes for transcripts from templates with the wild type element A, a fragment from the wild type U1+189 gene between the SalI site (at position 651 in the vector) and BamHI (at +189 in the U1 gene) was inserted between the SalI and BamHI sites of M13mp9 (42). To make probes for transcripts from templates with a replacement of element A, a BamHI fragment from -35/-8R1+189 that includes sequences between -393 and -35 and between -8 and +189 of the U1 gene was inserted into the BamHI site of M13mp9.

Transcription in X. laevis oocytes and nuclease S1 analyses

Five nanograms of plasmid DNA was injected into the nucleus of each of 15-25 centrifuged *Xenopus laevis* oocytes (43), and RNA was prepared from them about 22 hours later as described previously (5, 7). RNA from the equivalent of 0.5 oocytes was mixed with approximately 84,000 counts per minute of purified probe (in molar excess over the U1 RNA) in 15 µl hybridization buffer (80% formamide, 0.4M NaCl, 40mM PIPES [pH 6.4], 1mM EDTA) (44) and then was heated at 80-85°C for 10 minutes. Hybridization was then carried out at 50°C for at least 3 hours, after which 200 µl of 250mM NaCl, 50mM NaOAc (pH 4.6), 1mM ZnSO₄, 5% glycerol with 200 units nuclease S1 (1 min. assay for activity, Pharmacia) were added. The mixture was incubated for 1 hour at 22°C, and the nuclease S1 digestion was terminated by phenol extraction. Portions from each S1 experiment were analyzed on 0.4 mm thick gels containing 6% polyacrylamide (19:1), 7M urea, and 1×TBE (45). Molecular weight markers were derived from an MspI digest of pBR322 end-labeled with [γ -³²P]ATP and T4 polynucleotide kinase. Quantitation of the effects of each promoter mutation was done by scanning an appropriate exposure of the autoradiograms in Figure 3 with a Transidyne model 2955 densitometer (Transidyne General Corp., Ann Arbor, MI).

In the analysis of U1 genes with tandem coding regions and 3' end signals, each oocyte was co-injected with 5ng of plasmid DNA and 2.0 μ Ci [α - 32 P] GTP. After a 20-hour incubation RNAs were prepared from about 25 oocytes. RNAs containing U1 sequences were purified by hybrid selection (7) and analyzed by gel electrophoresis in 10% polyacrylamide (38.9:1.1), 7M urea, 1 $\times$ TBE.

RESULTS

We assayed the contribution of 5' flanking sequences of an snRNA gene on transcription initiation and on the recognition of its 3' end signal. This was done by injecting mutated forms of the human U1 gene HU1-1 into nuclei of *X. laevis* oocytes and analyzing the accumulated RNAs. The templates, shown in Figure 1, all contained an XhoI linker inserted between positions +27 and +28 in the coding region to allow us to distinguish between endogenous frog U1 RNAs and transcripts from these injected plasmids. U1 transcripts were identified by their ability to protect uniformly labeled single-stranded DNA probes against digestion by nuclease S1.

In these experiments only transcripts with 5' ends at position +1 (the normal cap site) were of interest. Other transcripts, originating at unknown upstream promoters, were identified by their ability to protect probes that extended beyond the 5' end of the normal transcripts (Figure 2). We defined the efficiency of correct 3' end formation as the fraction of the transcripts starting at the normal cap site that also had normal U1 3' ends near position +164 ('U1/preU1 3' ends'). As a control to ensure that the 3' ends at position +164 had been made in response to the 3' end signal, for each promoter mutant we also injected into a parallel set of oocytes the comparable template in which the 3' end signal had been inactivated by a partial deletion from downstream to position +185 (30).

When the template with the wild type promoter was assayed, most U1 transcripts extended from position +1 to the normal U1 3' end (lanes 1 and 3 in Figure 3), but about 20 percent of the transcripts from the normal cap site were elongated past the 3' end signal. Almost all the transcripts that initiated at position +1 became elongated when part of the 3' end signal was deleted to position +185 (lanes 2 and 4; reference 30). The bands representing read-through products from the +185 templates were split into doublets (204 and 193 nucleotides long), because the probe included flanking sequences 3' to position +185 that were not completely protected by transcripts from the templates deleted to +185. The large decrease in U1/pre-U1 3' ends from templates with this three base pair deletion probably results from changing three conserved base pairs, AGA, in the 3' end signal (46). The decrease in the level of these transcripts is not compensated completely by a corresponding increase in 3' elongated RNA because the 3' elongated snRNAs are less stable than those with correct 3' ends (27).

Figure 1. A human U1 RNA gene and deletions in its promoter. The top line shows the promoter elements of the human U1 RNA gene and the level of U1 synthesis in the absence of that element (20). Also included are potential binding sites for the following transcription factors: octanucleotide binding protein (OCTA), activator protein from adenovirus 2 major late transcription factor (MLTF), enhancer core from a variety of promoters (EnhC), and an snRNA conserved element binding factor (U1-B) (15). Elements D and B, also called the DSE and PSE, function like an enhancer and the TATA box, respectively, for the U1 gene. The upstream boundary of element C has not been mapped. The gray box between positions +27 and +28 represents a XhoI linker inserted to mark the transcripts from these templates. The black box on the right ('3' End') denotes the snRNA 3' end signal. The horizontal lines in the bottom two thirds of the figure indicate the structures of mutations made in the promoter. Thin lines indicate deleted sequences, open bars indicate promoter sequences that were replaced with a fragment from pBR322, and black bars indicate promoter sequences left intact.

Deletion of the 3' end signal also serves to confirm the identities of the various bands. Upon deletion of the 3' end signal, the bands that correspond to RNAs with 3' ends at position +164 become reduced in intensity, while those that represent read-through transcripts become more intense. Thus, the positions of the 5' ends of the transcripts can be deduced from the positions of the 3' ends and from the lengths of the probe that were protected.

Effects of deletions in individual promoter elements

We showed previously (30) that deletion of 5' flanking region sequences upstream of position -203 had little effect on the recognition of the 3' end signal. Sequences upstream of position -203 include element E (which is dispensable for efficient snRNA synthesis) (20), element D (the snRNA gene enhancer) and potential binding sites for several transcription factors (15). Thus these far upstream elements affect the accumulation of normal length U1 RNA by reducing the efficiency of initiation rather than by causing transcription to extend into the 3' flanking region. To maintain efficient transcription, most of the templates used below contained element D and associated factor binding sites extending upstream to position -231.

Although deletion of sequences between positions -129 and -138 significantly diminished the level of transcripts initiated at +1, recognition of the 3' end signal by correctly initiated snRNA transcription complexes was reduced only by about one quarter (lanes 7-10 of Figure 3). Thus element C also was not essential for 3' end formation. Likewise, sequences immediately adjacent to the 5' side of the coding region, in element A, were dispensable for recognition of the 3' end signal (lanes 19 and 20). Substitution of unrelated pBR322 sequences for the nucleotides between positions -8 and -35 did not affect the efficiency of U1/preU1 3' end formation.

Deletion of sequences just upstream of element B (between positions -105 and -80) influenced the efficiency of neither initiation nor 3' end formation (Figure 3, lanes 11 and 12). Deletion into the 5' side of element B (between positions -105 and -67) reduced transcription considerably but did not destroy the capacity of correctly initiated transcripts to terminate at the 3' end signal (lanes 13 and 14). Sequences between positions -80 and -67 were not absolutely required for recognition of the 3' end signal since templates lacking these sequences yielded correctly terminated U1 RNA only in response to an intact 3' end signal. The template with a deletion in the 3' end signal was incapable of bringing about U1/pre-U1 3' ends even on the few transcripts that initiated at +1. Unfortunately, the effect of these sequences on the efficiency of 3' end signal recognition could not be quantified because the portion of the DNA probe protected by correctly initiated but 3' elongated U1 RNAs comigrated with a 200 nucleotide long product protected by a transcript from an upstream promoter (lanes 13 and 14). (This artifact results in a 180 nucleotide long product in lanes 15 and 16.)

Deletion of all of element B (-105/-45) resulted in very low levels of U1 RNA that was initiated and terminated correctly. Since the amount of this transcript was almost unaffected by deletion of the 3' end signal, most of these normal 3' ends were not a result of recognition of the 3' end signal. Judging from the results with the -105/-67 deletion (described above), it is unlikely that the wild type and partially deleted 3' end signal would

Figure 2. Schematic representation of the nuclease S1 assay used to quantitate both the 5' and 3' ends of transcripts. The single-stranded probe, which included sequences complementary to U1 RNA, is indicated as a hatched bar on the bottom. It was uniformly labeled as detailed in Materials and Methods. Regions of the U1 transcripts that could hybridize to the probe are indicated as solid black lines.

Figure 3. Analysis of the U1 RNAs transcribed from the templates with deletions near or in individual promoter elements. Total RNA from oocytes injected with the templates indicated in Figure 1 were hybridized with the probe drawn in Figure 2. For each deletion in the promoter, two templates were injected: one with an intact 3' end signal (+), and one with the last three nucleotides of the signal deleted (-). The fraction of +1 starts that have 3' ends near position +164 ('U1/preU1') is indicated below each lane where the template had an intact 3' end signal. These fractions were determined by scanning densitometry of appropriate exposures of the indicated lanes. Transcripts from the template in which element A was replaced (lanes 19 and 20) were analyzed using a probe with the same substitutions between positions -8 and -35 as in the templates. Conditions for hybridizations and S1 nuclease digestions were as described in Materials and Methods. Autoradiography for lanes 3 and 4 was for 4 days without an intensifying screen, while lanes 1-2 and 5-20 were exposed for 14-15 days without intensifying screens. The vertical bars to the right of lanes 14 and 16 indicate the positions of artifacts caused by transcripts that initiated upstream and protected the probe to an AT-rich region near position +123; the upstream initiation of these transcripts is indicated by the shift in the size of the protected bands corresponding to deletions in the upstream region.

be able to generate the same level of U1/pre-U1 3' ends. A low level of transcripts that were initiated at the U1 cap site and elongated past the normal 3' end was suggested by the existence of a faint 200 nucleotide long nuclease S1 product. The scarcity of these transcripts indicates that the primary effect of a complete deletion of element B is to reduce the frequency of transcription initiation. Whether this deletion also prevents recognition of the 3' end signal is uncertain. These rare transcripts may have been some of the occasional U1 RNAs that normally extend past the 3' end signal.

Thus, every template that supported correct initiation of U1 RNA synthesis also had its 3' end signal recognized.

Element B alone is not sufficient for synthesis of U1 RNA

In an attempt to test whether element B alone was sufficient for both initiation and termination of transcription, we replaced all sequences upstream of position -105 and between positions -8 and -35. Surprisingly, this template, which included the region between positions -105 and -35, was transcriptionally inactive (Figure 4, lanes 1 and 2) (although the plasmid DNA did support transcription from promoters in pBR322, i.e., upstream starts). Thus element B alone is not sufficient for initiation of snRNA transcription. Consequently, we are unable to determine if element B alone is sufficient for recognition of the snRNA 3' end signal.

Since the other regions can be deleted individually without total disruption of transcription, they must include one or more regions that are functionally redundant for initiation of transcription. Likewise, because deletion of more than one of these regions also results

Figure 4. Analysis of U1 RNAs transcribed from templates with large deletions in the promoter. Total RNA from oocytes injected with the templates drawn on the bottom two lines of Figure 1 were hybridized with a form of the probe drawn in Figure 2 in which the same replacement had been made in element A. For each deletion in the promoter two templates were injected: one with an intact 3' end signal (+), and one with the signal deleted (-). The templates in lanes 1 and 2 contained U1 coding and flanking sequences from position -105 to -35 and from -8 to +189 (or +185) whereas those in lanes 3 and 4 contained sequences only from position -8 to +189 (or +185). Fragments protected by RNA from the upstream starts in the A-D deletions (lanes 3 and 4) have faster mobilities than fragments protected by RNAs from the corresponding starts in the deletion of elements A, C, and D (lanes 1 and 2) because the probe was not complementary to as much of the promoter region.

in no transcription from the normal cap site (data not shown), it is formally possible that elements A, C, and D include a redundant sequence that is required for 3' end formation.

Upstream starts

The abundant transcripts evident at the top of Figure 4 were initiated in vector sequences since they were present even when the template contained no U1 5' flanking sequences (lanes 3 and 4) or when the U1 gene was not fully functional (data not shown). Curiously, mutation of the snRNA 3' end signal led to elongation of some of these pBR322-promoted RNAs, indicating that their 3' ends were generated in response to this signal.

Deletion of element B (Figure 4) or other snRNA promoter elements (not shown) had no apparent effect on the frequency of ends generated by the snRNA 3' end signal in either population of pBR322-promoted RNAs. Thus, we conclude that the mere presence of this promoter element upstream of the 3' end signal is not sufficient for snRNA 3' end formation. Instead, transcription must be initiated at an snRNA-type promoter using, among other transcription signals, element B.

The 3' end signal closest to the promoter is used preferentially

To test whether the position of the 3' end signal affects its use, we constructed a set of U1 genes with tandem copies of the wild type (U1) and maxi-U1 (Bi33) coding region

Figure 5. Transcription of U1 genes with tandem coding regions and 3' end signals. (A.) Schematic representation of the templates injected into oocyte nuclei. Bi33 includes a 33 base pair tandem duplication at the 5' end of the coding region (5). Intact and partially deleted 3' end signals are indicated by black and white rectangles, respectively. (B.) Transcripts from templates drawn in (A.). RNAs containing U1 sequences were labeled with ^{32}P *in vivo* and purified by hybrid selection. Numbers in parentheses indicate the sizes of the transcripts, in nucleotides. Termination at the proximal 3' end signal seems more efficient than in experiments in which a linker has been inserted at the BclI site at position +27 (*cf.* Fig. 3; references 30, 46) because this insertion disrupts binding of a U1-specific protein which might be required for greater stability of the RNA with normal 3' ends (57–59, H.E.N.dV., unpublished results). The +185/Bi33 template appears to yield greater levels of transcription probably because the hybrid selection was more efficient on the read-through transcripts which had two copies of the U1 coding region.

each with part or all of the 3' end signal (Figure 5A). In all templates where the upstream 3' end signal was intact (U1 + 189/Bi33, U1 + 199/Bi33, and Bi33/U1 + 199) the upstream signal was preferred (Figure 5B, lanes 2–4). The downstream signal was favored only when the upstream signal was disabled (U1 + 185/Bi33) (lane 1). The downstream signal was functional since it directed 3' end formation of occasional read-through products (Figure 5B, especially lanes 2 and 4). Thus, the low frequency of 3' ends at the downstream site resulted from greater utilization of the upstream site, and not from the inactivation of the downstream site. Replacement of the downstream Bi33 coding sequence with the normal U1 coding sequence did not affect the preference for the upstream 3' end signal (Figure 5, lane 4).

DISCUSSION

The role of promoter elements in termination of transcription

In this paper we have shown that initiation and 3' end formation of snRNAs cannot be uncoupled. The deletions in the U1 promoter region that significantly reduced the level

of U1 RNA accumulation did so by reducing transcription initiation and not by preventing recognition of the 3' end signal. In all cases 50–80 percent of the transcripts initiated at the normal U1 cap site (at +1) also had U1/preU1 3' ends.

Deletion of element B, the functional equivalent of a TATA box for the snRNA gene, destroyed the ability of the template to direct synthesis from position +1. It remains possible that element B consists of multiple sites, each specifying a separate function of the snRNA transcription complex, such as the positioning of the cap site, the frequency of initiation events, or the ability of the RNA polymerase to recognize the 3' end signal.

The sensitivity to the snRNA 3' end signal by some transcription complexes that had initiated in pBR322 sequences (Figure 5, lanes 3 and 4) could reflect either of two phenomena. It is possible that adventitious pBR322 promoters may contain elements resembling those of snRNA promoters which also could cause recognition of the U1 3' end signal. Additionally, we and others have noted that a significant fraction of the transcription initiated at mRNA promoters respond to the 3' end signal (3, 30). In the case of U1 RNA made from a thymidine kinase promoter, as much as 20 percent of the transcripts stopped at the snRNA 3' end signal (30). We note that the snRNA 3' end signal does not act as an 'intrinsic termination site' of the kind observed on naked DNA with highly purified RNA polymerase II (47–49, and C. Kane, personal communication).

Roles of promoter elements in initiation of transcription

The snRNA promoter elements analyzed in this study were mapped originally by their effects on the accumulation of a discrete species of U1 RNA (20). The presence of such a band of RNA in a gel requires both efficient initiation and precise termination. The data in those and other studies (cited in reference 18) were interpreted on the assumption that the mutations would affect initiation rather than termination. We have demonstrated here that this premise was valid since each of these elements affect initiation of transcription to a much greater extent than 3' end formation. This assumption about the effect of promoter deletions was also justified in experiments on a U2 gene using primer extension, a technique that also does not depend on synthesis of a discrete band of RNA (11, 50).

Surprisingly, templates containing only an intact element B alone were unable to support snRNA synthesis. Previously, we considered the other elements as having ancillary functions which would stimulate transcription but that were not absolutely required. Presumably, interactions between sequences in element B and factors that bind to them, while highly specific, are too weak to support initiation of transcription without other DNA-protein interactions in the 5' flanking region. In this regard, it is interesting that templates with element B and only one of the other elements (A, C, or D) are inactive (data not shown), but templates with element B plus any two other elements are active (Figure 3).

Upstream starts

Disruption of the 5' flanking region of the U1 gene appeared to increase the level of transcription from regions in pBR322 sequences into the U1 coding region (*q.v.* reference 27). Comparable upstream starts have also been detected upon injection of a human U2 gene, a chicken ovalbumin gene, and pBR322 alone into frog oocytes (48–50). The increase in such transcripts upon deletion of element D (the U1 gene enhancer) could well explain the earlier report by Hernandez and Weiner (3) in which the absence of 5' end mapping led to the erroneous conclusion that the enhancer of a U2 RNA gene was required for recognition of the 3' end signal. More recently, Hernandez and Lucito (53), utilizing a probe that resembles the one used by us, have modified their conclusions to agree with our observations (Figure 3, and reference 30).

Possible mechanisms for coupling of termination to initiation

The inability to uncouple recognition of the snRNA 3' end signal from correct initiation of transcription indicates that termination at the 3' end signal is probably an intrinsic property of a special type of complex that transcribes snRNA genes. This obligate recognition of a termination signal would be analogous to the way that RNA polymerase III recognizes oligo-dA and flanking sequences in the template strand as a termination signal (54). The incorporation of these properties into snRNA-specific transcription complexes is made likely by the unusual features of snRNA promoters (reviewed in references 18 and 19), including the sequence and location of the TATA-like element (element B), the specificity of snRNA enhancers for activation of transcription of snRNAs but not mRNAs (13, 21–23), and the difficulty in obtaining *in vitro* transcription of these genes (4, 7). Furthermore, the promoters for snRNAs U1-U5 share similar proximal and distal elements with U6 RNA genes, which are normally transcribed by RNA polymerase III (18). It is significant that the polymerase specificity and the responsiveness to the respective 3' end signals of both classes of snRNA genes can be interchanged by small alterations in promoter sequences (55, 56).

The template probably does not fold back on itself to allow simultaneous binding of a factor to 5' transcription signals and the 3' end signal. The preferred use of the 3' end signal closest to the promoter suggests that the recognition of the 3' end signal occurs as the transcription complex proceeds over that signal. If selection of the termination site occurred at the time of initiation of transcription, the upstream and downstream 3' end signals probably would have been recognized with equal frequency. Significantly, the few correctly initiated transcripts that extended past the wild type 3' end signal terminated at the downstream signal, indicating that read-through at the upstream signal had occurred randomly among otherwise equivalent snRNA transcription complexes rather than as a result of the absence of a termination factor (or the presence of an anti-termination factor) in a subpopulation of transcription complexes.

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