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Transcription Terminator-Like Element within a *Saccharomyces cerevisiae* Promoter Region

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We analyzed a cloned fragment of the yeast *URA3* promoter region that contains a sequence of DNA capable of functioning as a highly efficient transcription terminator. BAL 31 deletions have shown the signal for the transcription termination activity is ≤110 base pairs and resides between bases 45 and 155 upstream of the *URA3* primary ATG codon at base 227. In our in vivo assay system, the DNA fragment is able to terminate transcripts very efficiently in either orientation. The terminated transcripts bind to oligodeoxythymidylate cellulose columns and promote the synthesis of full-length cDNAs, suggesting that the transcripts are polyadenylated. The 110-base-pair region contains no sequence resembling terminator consensus sequences described by Zaret and Sherman (K. S. Zaret and F. Sherman, Cell, 28:563–573, 1982) or Henikoff and Cohen (S. Henikoff and E. H. Cohen, Mol. Cell. Biol., 4:1515–1520, 1984). We discuss the possible physiological relevance of this sequence to bona fide termination of transcription and to *URA3* regulation in *Saccharomyces cerevisiae*.

In eucaryotic genes studied so far, with the exception of histone genes, small nuclear RNAs, and most yeast polII genes, RNA polymerase II transcribes through 3'-terminal polyadenylation sites(s) and terminates up to 1,000 nucleotides beyond the DNA sequences coding for the 3' mRNA termini (6, 9, 19, 26). In *Saccharomyces cerevisiae*, little is known yet about the structure and function of transcription terminators; we cannot yet differentiate between transcription termination signals versus processing sites. The 3' ends of yeast mRNAs appear to lie closer to the termini of the coding region (31, 32). In *S. cerevisiae*, precise termination events appear to be important. For example, alterations in the endpoint of yeast *CYC1* mRNA not only have major effects on *CYC1* mRNA turnover, but also severely affect the translational efficiencies of these mRNAs even though they are polyadenylated (32). Virtually all mRNAs in *S. cerevisiae* are polyadenylated, including histone mRNAs (8). Unlike higher eucaryotes in which polyadenylation may be directly coupled to 3' end cleavage and processing of mRNA, polyadenylation in *S. cerevisiae* may be coupled to actual transcription termination (31, 32).

The termini of several yeast genes have recently been analyzed for possible transcription termination signals. From mutational analysis of the *CYC1* gene, Zaret and Sherman (31) have proposed a consensus sequence of TAG. . .TAGT. . .(A-T rich)TTT as being a terminator signal causing transcription to occur a short distance downstream. BAL 31 deletion analysis of a *Drosophila* terminator cloned in *S. cerevisiae* has led to an alternative consensus sequence TTTTTATA being proposed as a transcription terminator for yeast genes in which the terminal mRNA sequence is CAAG$\overset{T}{C}$TTTG (10, 11). However, Zaret and Sherman (31) and Henikoff and Cohen (10) suggest that many yeast genes do not contain the 3'-terminal sequences proposed by either group. Further confusion over what constitutes a yeast transcription terminator has arisen since sequences very similar to the consensus sequence of Zaret and Sherman (31) are found both within the coding region and upstream of other genes (29).

The continued lack of understanding of what constitutes a yeast transcription terminator(s) and in how many locations yeast transcription terminator elements appear prompted us to search for and investigate regions of DNA in *S. cerevisiae* that have the capacity to function as transcription terminators. During our investigation, we isolated a fragment of *URA3* DNA capable of highly efficient transcription termination. Throughout this paper we will use the phrase transcription terminator loosely because of the possibility that a particular region could encode: (i) an actual signal for termination to occur a short distance away, (ii) an RNA processing site, (iii) a polyadenylation site, or (iv) a combination of the above. This paper describes the detailed analysis of the transcription termination activity associated with the yeast *URA3* promoter region.

MATERIALS AND METHODS

***Saccharomyces cerevisiae* strains and media.** *S. cerevisiae* DBY745 (α *ade1 ura3-52 leu2-100 leu2-112*) was obtained from David Botstein. Cells were grown in either YEP medium (per liter; 10 g of yeast extract, 20 g of peptone, and 20 g of glucose) or modified MVA medium (per liter; 6.7 g of yeast nitrogen base without amino acids, 10 g of succinic acid, 6 g of NaOH supplemented with adenine and leucine, and either 20 g of galactose or 20 g of glucose).

DNA manipulations and DNA sequencing. All restriction enzymes, T4 DNA ligase, *Escherichia coli* DNA polymerase Klenow fragment, synthetic linkers, and BAL 31 nuclease were purchased from either New England Biolabs or Boehringer Mannheim. DNA sequences were determined by the chemical cleavage method of Maxam and Gilbert (17). Analysis of DNA sequences and homology searches were performed with the aid of the Intelligenetics ⟨SEQ⟩ computer program. Plasmid constructions were performed by standard methods (15).

Yeast transformations. Yeast cells (100 ml) were grown to a concentration of 2×10^7 cells per ml. The cells were harvested and washed once with 5 ml of TE (10 mM Tris [pH

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7.5], 1 mM EDTA) and once with 5 ml of lithium acetate (LioAc) solution (10 mM Tris [pH 7.5], 1 mM EDTA, and 0.1 M LioAc). The cell pellet was suspended in 1 ml of LioAc solution and incubated for 1 h at 30°C. Competent yeast cells were either stored for ≤2 days at 4°C or used immediately as follows. Cells (0.1 ml) were added to 15 μl of plasmid DNA (≤1 μg DNA) plus denatured salmon sperm DNA (15 μl of 3-mg/ml solution) added as a carrier. After incubation for 30 min at 30°C, 0.7 ml of polyethylene glycol solution (40% polyethylene glycol 3350, 0.1 M LioAc, 10 mM Tris [pH 7.5], 1 mM EDTA) was added, and incubation was continued for 1 h at 30°C. The cells were heated at 42°C for 5 min and pelleted for 4 s in an Eppendorf microfuge. The cell pellet was washed twice with 0.5 ml of TE, followed by centrifugation for 2 s. The final cell pellet was suspended in 0.1 ml of TE and plated onto selective agar plates.

RNA extraction and Northern gel. For large RNA preparations, 5 ml of cycloheximide solution was added to 1 liter of cells at 4×10^7 cells per ml at 10 min before harvesting. After being pelleted, the cells were suspended in 3 ml of RNA buffer (50 mM Tris [pH 6.8], 2 mM EDTA, 50 μg of cycloheximide per ml) and transferred to a 30-ml Corex tube. The cells were ruptured by vortexing six times for 20 s each in the presence of 0.45-mm glass beads. Sodium dodecyl sulfate (0.1 volume, 10% solution) and 0.1 volume of 2 M NaAc (pH 5.2) were added, followed by four extractions with phenol-chloroform-isoamylalcohol (50:50:1). The final aqueous solution was precipitated by the addition of 0.1 volume 2 M NaAc (pH 5.2) and 2.5 volumes of 95% ethanol.

For RNA minipreps, 2 ml of cycloheximide (200 μg/ml) were added to 10 ml of cells at 4×10^7 cells per ml. The cells were pelleted, suspended in 0.5 ml of RNA buffer and transferred to an Eppendorf tube on ice. Two Eppendorf lid-fulls of 0.45-mm glass beads were added, and the cells were vortexed four times for 20 s each. Then, 50 μl of 3 M NaAc, 50 μl of 10% sodium dodecyl sulfate, and 750 μl of water-saturated phenol were added. The solution was extracted three additional times with phenol. The RNA was precipitated by the addition of 50 μl of 2 M KAC (pH 5.2) and 1 ml of 95% ethanol.

Formaldehyde agarose gel electrophoresis was performed as previously described (30) and Northern transfers were performed as described by Thomas (25).

Polyadenylic acid [poly (A)]-containing RNA was prepared by oligodeoxythymidylate [oligo(dt)] cellulose chromatography as described by Aviv and Leder (2).

Hybridizations. Northern filters were prehybridized for 2 h at 42°C in 50% formamide, 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt solution, 50 mM $NaPO_4$ (pH 6.5), and 200 μg of denatured salmon sperm DNA per ml. Hybridization was performed for 12 to 16 h at 42°C in 50% formamide, 5× SSC, 20 mM $NaPO_4$, (pH 6.5), 10% dextran sulfate, 1× Denhardt solution, 100 μg of denatured salmon sperm DNA per ml, and labeled DNA probe at $\geq 2 \times 10^5$ cpm/ml of hybridization buffer.

Filters were washed two times at room temperature for 5 min in 2× SSC plus 0.1% sodium dodecyl sulfate and then two times for 20 min each at 42°C in 0.2× SSC plus 0.1% sodium dodecyl sulfate. Filters were exposed to film at −70°C with DuPont Cronex Lightening Plus intensifying screens.

Synthesis of synthetic oligomers. Oligomers were synthesized by the phosphorimidite procedure with an Applied Biosystems 380A DNA synthesizer.

Other procedures. M13 double-mismatch mutagenesis was performed by the double primer method of Norris et al. (20). Nick translations were performed by the method of Maniatis et al. (16). First-strand cDNA synthesis was performed as described in Maniatis et al. (15). β-galactosidase enzyme activity was measured as previously described (29).

RESULTS

A 450-bp DNA fragment from a yeast plasmid terminates transcription. We initiated a search for regions of yeast DNA that are capable of functioning as transcription terminators in *S. cerevisiae*. Our transcription termination assay was a functional assay in vivo in which we tested DNA fragments for the ability to prematurely terminate *GAL7* transcription. Although we were looking for any DNA fragment that has the capacity to function as a terminator, we initially focused our attention on the *GAL10* gene from plasmid pBD6.

Plasmid pBD6 (29) contained the amino terminal 555 base pairs (bp) of the *GAL7* gene fused to *lacZ*, the carboxyl terminal 407 bp of the *GAL10* gene, a 730-bp noncoding region between *GAL10* and *GAL7*, the *URA3* gene, and the yeast 2μ replicon (Fig. 1). Since we planned to isolate a 450-bp fragment of *GAL10* DNA between the *Taq*I and *Nco*I restriction sites from the plasmid pBD6 (Fig. 2) and our assay was for function, we simultaneously isolated a 450-bp *Taq*I to *Nco*I fragment from plasmid pBD6-R. The plasmid pBD6-R was identical to plasmid pBD6 except that it contained a substitution of *URA3* amino-terminal DNA for *GAL10* DNA (Fig. 1). We anticipated obtaining a functional *GAL10* terminator but could also identify a region from *URA3* if that DNA had the capacity to function as a yeast transcription terminator in vivo. In our first assay of these cloned 450-bp DNA fragments for terminator activity, one cloned fragment was ligated in either orientation (GA11 and GA23; Fig. 3A) into the unique *GAL7 Bam*HI site in plasmid pBD6 and then used to transform *S. cerevisiae* DBY745. *S. cerevisiae* cultures containing either GA11 or GA23 plasmids were grown in MVA medium containing either glucose (uninduced) or galactose (induced). Total yeast cellular RNA was isolated, fractionated on a formaldehyde-agarose gel, transferred to a nitrocellulose filter, and hybridized to a 500-bp ^{32}P-nick-translated DNA probe specific for the *GAL7* coding region. When the cells were grown on galactose, the probe DNA hybridized to the 1,350-nucleotide genomic *GAL7* RNA (Fig. 4). In addition, cells that contained plasmid GA11 showed a strongly hybridizing 900-nucleotide RNA. Cells that contained plasmid GA23 showed a strongly hybridizing 700-nucleotide RNA. The 1350-, 900-, and 700-nucleotide transcripts appeared only when galactose was the carbon source. The data are consistent with a model in which the truncated *GAL7* RNA transcripts from plasmid GA11 and plasmid GA23 terminate within a similar region at one end of the 450-bp fragment (Fig. 3). The 900-nucleotide-terminated RNA observed in Fig. 4 (plasmid GA11) could be produced by the 555-nucleotide *GAL7* RNA plus the 300-nucleotide terminator RNA before truncation (Fig. 3). With the terminator DNA fragment present in the opposite orientation (plasmid GA23), the 700-nucleotide-terminated RNA (Fig. 4) could be produced by the 555-nucleotide *GAL7* RNA plus the 100-nucleotide terminator RNA before termination. In both cases, the model allows for the presence of a poly(A) tail of approximately 50 nucleotides average length as is suggested for yeast mRNAs (18, 24).

Complete signal for termination at one end of the 450-bp fragment. To determine the location of the transcription termination signal(s) within the 450-bp fragment, we created a series of deletions of the terminator fragment. In both plasmid GA11 and plasmid GA23, deletions were created in

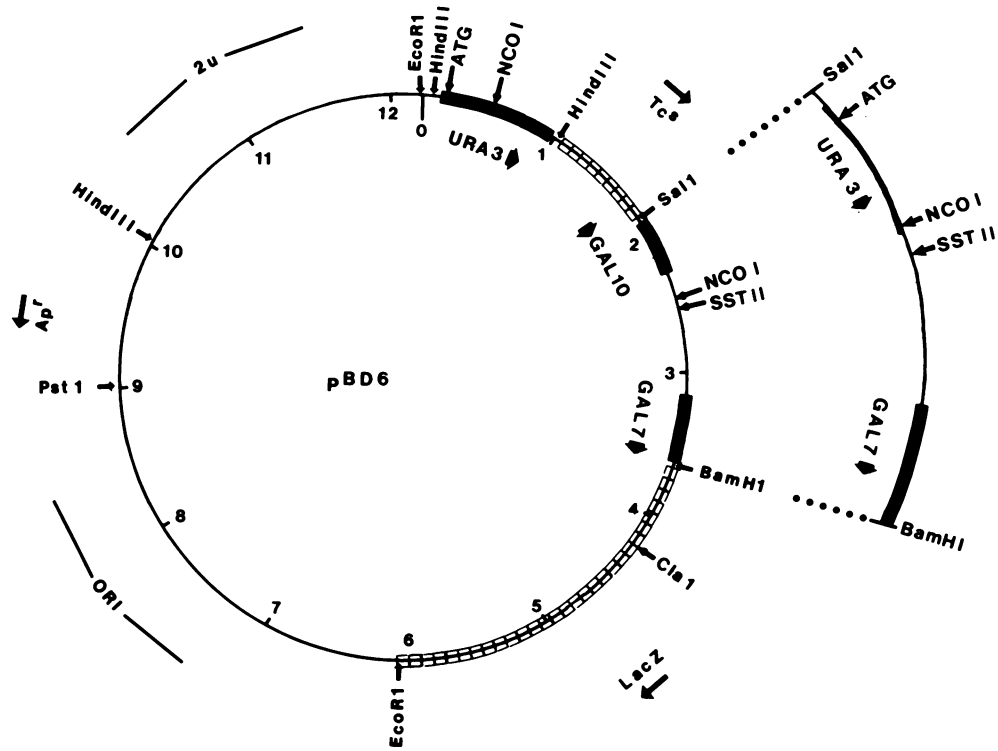

FIG. 1. Structure of plasmids pBD6 and pBD6-R. The original plasmid pBD6 (constructed by Barbara Dunn) contains an insertionally inactivated tetracycline gene (Tc^s) with the *URA3* gene located within the promoter region; the ampicillin resistance gene (Ap^r); the bacterial origin of replication (Ori); and the B fragment of the yeast 2μ replicon (2μ). The amino-terminal portion of *GAL7* is fused inframe to *LacZ*, and *GAL10* is the carboxyl terminus of *GAL10* (see text). The insert denotes the differences between plasmid pBD6 and pBD6-R. The sizes of the two plasmids are essentially identical.

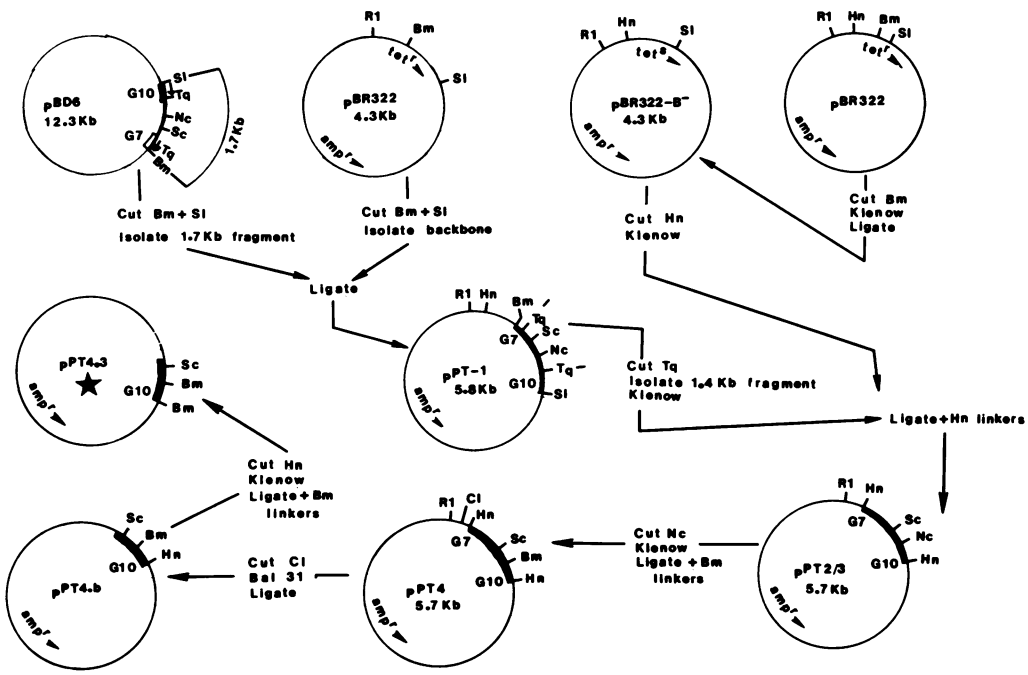

FIG. 2. Scheme used to isolate the 437-bp terminator fragment. The following abbreviations refer to restriction sites: Bm (*Bam*HI), Cl (*Cla*I), Rl (*Eco*RI), Hn (*Hin*dIII), Nc (*Nco*I), Sl (*Sal*I), Sc (*Sac*II), Tq (*Taq*I). The plasmid, pPT 4.3 (denoted by star), contains the 437-bp terminator fragment bounded by *Bam*HI linkers. The abbreviations *G7* and *G10* refer to *GAL7* gene segment and *GAL10* (pBD6) or *URA3* (pBD6-R) gene segment, respectively. In this scheme only, the beginning plasmid pBD6 refers to a mixture of pBD6 DNA and pBD6-R DNA. The *Taq*I digest was a partial digest, whereas all other restriction digests were complete.

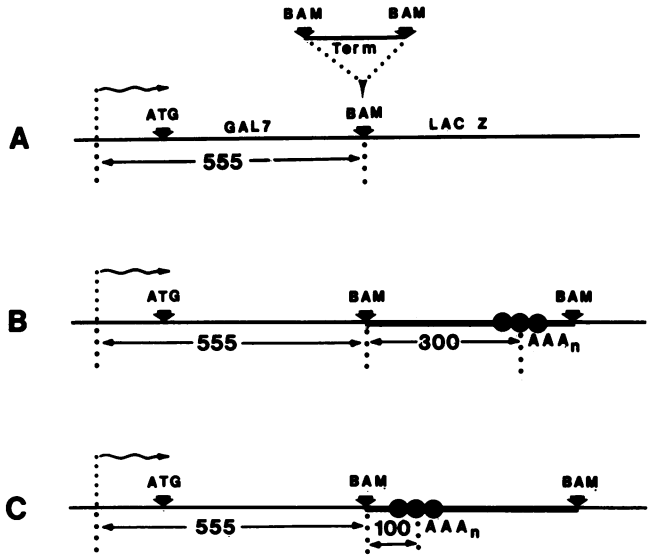

FIG. 3. Model for terminator activity. (A) The *GAL7/lacZ* *Bam*HI junction region from original plasmid pBD6 into which the 437-bp terminator fragment was ligated in either orientation. (B) Plasmid GA11 contains the 437-bp terminator ligated in the orientation shown. (C) Plasmid GA23, with the terminator in the opposite orientation.

vitro by digestion with BAL 31 nuclease from the unique *Cla*I site within *lacZ* (Fig. 1). Ligations were performed in the presence of *Cla*I linkers to mark each deletion endpoint. In all cases, the ends of the deletions were determined by DNA sequencing. RNAs extracted from yeast cells containing these deletion plasmids were examined by Northern analysis for the presence or absence of the 700-nucleotide *GAL7* RNA (plasmid GA23 deletions) and of the 900-nucleotide *GAL7* RNA (plasmid GA11 deletions). There is no change in termination patterns for deletions extending up to ΔGA11-41 (41-bp deletion within the terminator fragment) from one direction and up to ΔGA23-284 (284-bp deletion of terminator DNA) from the other (Fig. 5). However, additional deletions, e.g., ΔGA11-220 (Fig. 5, lane J) and ΔGA23-437 (lane E) eliminated the transcription termination activity. These data show that the ability to terminate in both orientations is dependent upon a ≤110-bp region between bp 40 and bp 155 (Fig. 5B). We have not determined the origin of the hybridizing RNAs which are observed to migrate slower than 28 s (Fig. 5).

450-bp termination fragment derived from *URA3*. We sequenced the entire 450-bp terminator fragment and corresponding deletions and determined that the sequence showed complete homology to the amino-terminal end of the *URA3* gene (Fig. 6). The 110-bp region of *URA3* that is capable of bidirectional transcription termination ends 72-bp upstream of the *URA3* ATG and lies just upstream of the three major *URA3* RNA transcription start sites as determined by Rose and Botstein (1983). It is interesting to note that this 110-bp region contains no sequence resembling either the termination sequences described by Zaret and Sherman (31) or by Henikoff et al. (10, 11).

GAL7 transcripts terminated by bidirectional 110-bp fragment are polyadenylated. To determine whether the GA11- and GA23-terminated *GAL7* transcripts were polyadenylated, the total RNA extracted from galactose-grown cells was subjected to chromatography on oligo(dT) cellulose columns. The eluted RNAs were then analyzed by Northern

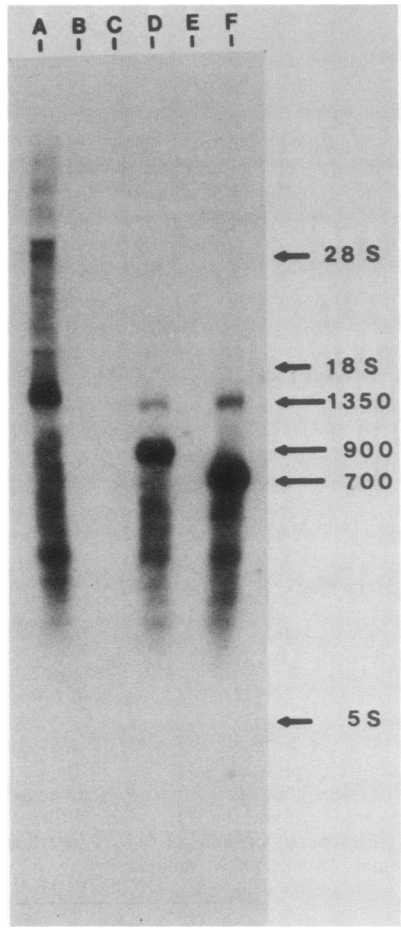

FIG. 4. Detection of in vivo-terminated *GAL7* transcripts. Northern hybridization of total yeast RNA extracted from cells grown on glucose (lanes B, C, and E) or galactose (lanes A, D, and F), and containing plasmid pBD6 (lanes A and B), plasmid GA11 (lanes C and D), or plasmid GA23 (lanes E and F). The RNAs were hybridized with a *GAL7* probe. The 500-bp *GAL7* probe was obtained by cutting plasmid pBD6-Xho with *Xho*I and *Bam*HI. The *Xho*I site is located 4 bp in front of the *GAL7* ATG, while the *Bam*HI site is located 500 bp downstream of the *GAL7* ATG at the *GAL7-lacZ* fusion junction. The 28 s and 18 s rRNA and 5 s (tRNA) migration patterns are shown. Numbers indicate: 1350, genomic GAL7 RNA; 900, GA11-terminated *GAL7* RNA; GA23-terminated *GAL7* RNA.

hybridization to *GAL7* DNA probes. The oligo(dT) cellulose-bound fractions contained virtually all of the GA11- and GA23-terminated *GAL7* RNAs (Fig. 7).

To determine whether retention of GA11- and GA23-terminated RNAs on oligo(dT) cellulose was due to polyadenylation, we asked whether these RNAs could act as oligo(dT) primer-dependent templates for reverse transcriptase. (The GA23 RNAs could contain a large transcribed stretch of U's [57% present between bases 1 and 70, see Fig. 6] depending upon where truncation occurs in relation to the position of the terminator. Thus, it is possible that GA23 RNA could yield a less than full-sized oligo(dT) primer-independent cDNA if the terminal poly(A) tract base paired with the internal polyuridylic acids. This could not occur in the case of GA11.) We looked for the presence of full-size cDNA transcripts by fractionating the cDNA on formaldehyde agarose gels as described in Fig. 8, transferring the

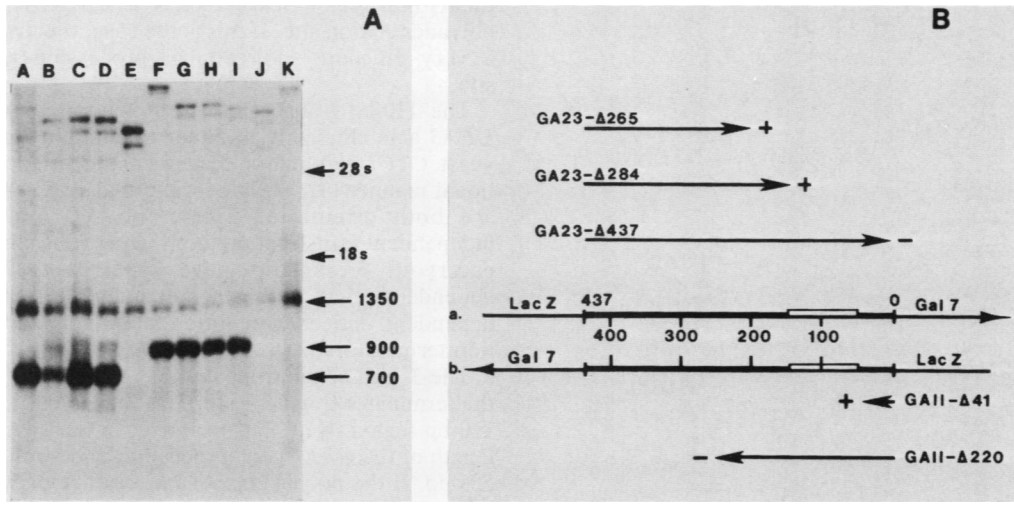

FIG. 5. Effect of BAL 31 deletions on terminator activity. RNA was extracted from cells grown on galactose and analyzed by Northern hybridization using a nick-translated, *GAL7*-specific probe. (A) Lanes A through E correspond to RNA extracted from cells containing untreated plasmid GA23, GA23Δ300, GA23Δ265, GA23Δ284, and GA23Δ437, respectively. Lanes F through J correspond to RNA extracted from cells containing untreated plasmid GA11, GA11Δ1, GA11Δ10, GA11Δ41, and GA11Δ220, respectively. Lane K is pBD6 control RNA. (B) The deletions shown in A delimit a 110-bp region (designated as open rectangle) within the 437-bp fragment which is sufficient for terminator activity.

cDNA to nitrocellulose, and then probing the filter with a synthetic 60-mer probe complementary to the 20-nucleotide untranslated leader of *GAL7* plus the first 40 nucleotides of the coding region. Full-length cDNA (900 nucleotides) was obtained from GA11-truncated RNA, and the synthesis was totally dependent upon the addition of exogenous oligo(dT) primer (Fig. 8). The full-length cDNA obtained from GA23 mRNA (700 nucleotides) was also dependent upon the addition of exogenous primer, although two smaller *GAL7* cDNAs were also obtained in the absence of exogenous oligo(dT) primer as expected. We conclude that both GA23 and GA11 RNAs are good templates for cDNA synthesis when primed with oligo(dT), and thus are polyadenylated.

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        AA  T       20      TT  30          40          50          60
AGCTTTTCTT TGCAATTCAT CATTGGTTTT TTATTCTTTT TTTTGATTTC GGTTTCTTTG
        **  *           **

        70          80          90          100         110         120
AAATTTTTTT GATTCGGTAA TCTCCGAACA GAAGGAAGAA CGAAGGAAGG AGCACAGACT
                                 ●●●●● ●●●●●●●●●● ●●●●●●●●●● ●

        130         140         150         160         170         180
TAGATTGGTA TATATACGCA TATGTAGTGT TGAAGAAACA TGAAATTGCC CAGTATTCTT

        190         200         210         220         229
AACCCAACTG CACAGAACAA AAACCTGCAG GAAACGAAGA TAAATCATC TCG AAA GCT
                                                    Met ser lys ala

                250                                             283
ACA TAT AAG GAA CGT GCT GCT ACT CAT CCT AGT CCT GTT GCT GCC AAG CTA
thr tyr lys glu arg ala ala thr his pro ser pro val ala ala lys leu

                301                                             334
TTT AAT ATC ATG CAC GAA AAG CAA ACA AAC TTG TGT GCT TCA TTG GAT GTT
phe asn ile met his glu lys gln thr asn leu cys ala ser leu asp val

                352                                             385
CGT ACC ACC AAG GAA TTA CTG GAG TTA GTT GAA GCA TTA GGT CCC AAA ATT
arg thr thr lys glu leu leu glu leu val glu ala leu gly pro lys ile

                403                                             436
TGT TTA CTA AAA ACA CAT GTG GAT ATC TTG ACT GAT TTT TCC ATG CGGATCCG
cys leu leu lys thr his val asp ile leu thr asp phe ser met
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FIG. 6. DNA sequence of the 437-bp terminator fragment. The major ATG methionine start codon for *URA3* RNA is shown at bp 230. The 110-bp region from bp 45 to bp 155 that contains the bidirectional transcription terminator activity is underlined and printed in large letters. The 5 bases which differ when our 437-bp *URA3* DNA sequence is compared to the published *URA3* gene sequence (23) are marked with an asterick (*), with the published base written above. The *Nco*I site of *URA3* as described in the text is at bp 437, whereas the digested *Taq*I site is just before bp 1. The direct repeat is denoted by closed circles. The region of dyad symmetry is from bp 126 to bp 140.

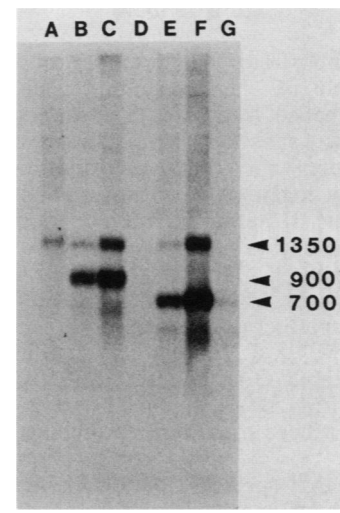

FIG. 7. Northern hybridization of oligo(dT)-bound RNAs. Total RNA extracted from galactose-grown cells containing either plasmid GA23 or GA11 was enriched for poly(A)-containing RNA by oligo(dT) cellulose chromatography. For the Northern gel, 0.9 μg of either total or oligo(dT)-bound RNA was used in each lane. The Northern filter was hybridized with nick-translated, *GAL7*-specific DNA. Lanes A, pBD6 control RNA; B, GA11 total RNA; C, GA11 oligo(dT) RNA; D, GA11 flow-through RNA; E, GA23 total RNA; F, GA23 oligo(dT) RNA; and G, GA23 flow-through RNA.

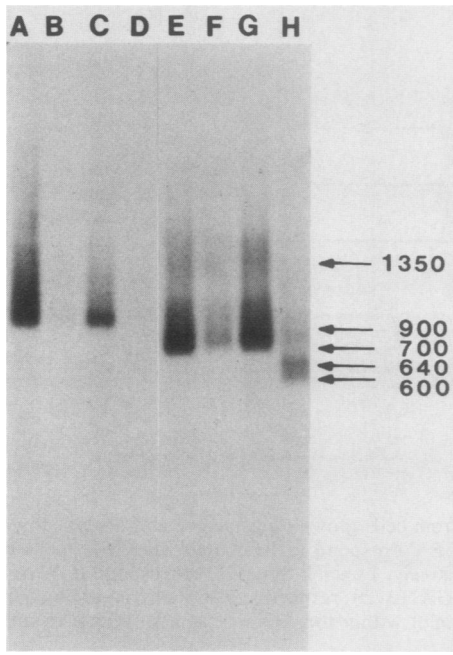

FIG. 8. Detection of cDNA synthesized from truncated *GAL7* RNA. Single-stranded cDNA was synthesized in the presence or absence of oligo(dT) primer from oligo(dT) cellulose-enriched GA11 RNA and GA23 RNA as described in the text. The cDNAs were size separated on a 1% agarose gel and hybridized to a synthetic probe identical to the *GAL7* RNA region containing the 20-nucleotide untranslated leader plus the first 40 nucleotides of the *GAL7*-coding region. Each lane contained either 0.5 or 2.0 μg of (as noted) cDNA (lanes): A + C, GA11 cDNA, 2 μg; B, GA11 cDNA, 0.5 μg; D, GA11 cDNA control, no primer added, 2 μg; E + G, GA23 cDNA, 2 μg; F, GA23 cDNA, 0.5 μg; H, GA23 cDNA control, no primer added, 2μg.

DISCUSSION

We have demonstrated the presence of a yeast DNA sequence capable of efficient, bidirectional transcription termination sequence located just upstream of the three major mRNA start sites for *URA3*. The terminator activity was not dependent upon its location within a gene but worked at many locations (data not shown). The 110-bp RNA polymerase II terminator region demonstrated no discernible homology to either the consensus sequence of Zaret and Sherman (31, 32) or to that of the Henikoff et al. (10, 11). The sequence of the 110-bp region (Fig. 6) shows one region of imperfect dyad symmetry [from bp 126 to bp 140 $^{TGGTATATATACGCA}_{ACCATATATATGCGT\text{-}5'}$]. There is also a ca. 15-bp direct but overlapping repeat [5′ GAACAGAAGGAAGGA] from bp 86 to bp 100 and from bp 98 to bp 111. It is unknown yet whether either of these structures are involved in terminator activity.

It is apparent that our GA11- and GA23-terminated transcripts are polyadenylated as they are good templates for oligo(dT) primer-dependent cDNA synthesis. The poly(A) tract cannot be less than 5 to 30 nucleotides in size since shorter poly(A) tracts are not retained on oligo(dT) cellulose (4). Our data are consistent with the hypothesis proposed by Zaret and Sherman (31, 32) that transcription termination and polyadenylation events are coupled in *S. cerevisiae*. However, we cannot rule out the possibility that RNA polymerase II proceeds beyond our 110-bp signal and that the 110-bp region serves solely as an RNA processing or polyadenylation site. If this is the case, then we have located a very efficient, bidirectional processing-polyadenylation site.

The 110-bp transcription terminator region upstream of *URA3* acts efficiently in either orientation. In contrast, the yeast *CYC1* terminator appears to function in a unidirectional manner (32). It is possible that in *S. cerevisiae* there are both orientation-dependent (32) and orientation-independent transcription terminators (this paper). Although eucaryotic terminators have appeared to be orientation dependent (7), *E. coli* genes demonstrate both orientation-dependent and efficient orientation-independent transcription terminators (21).

The 3′ end of the truncated RNA, in regard to location of the terminator fragment, appears to be close to or within the 110-bp region itself. For example, in the case of GA23, the length of the *GAL7* gene preceeding the terminator region is 555 bp. If the poly(A) tract is an average of 50-bp in length, then approximately 100 bp of the original 437-bp terminator fragment in GA23 must be transcribed before termination. This would place the site of actual termination approximately at the proximal end of the 110-bp terminator region (see Fig. 4 and 6).

The presence of transcription terminators in front of genes may not be a rare event. For example, the *Xenopus laevis* rRNA initiation site is preceeded by an oligonucleotide which has been suggested to have the features of a transcription termination site (24a). In bacteria, most of the amino acid biosynthetic operons have transcription attenuators (terminators) in their leader regions (12). In addition, the yeast *LEU2* gene may be regulated through a transcription attenuation mechanism (1, 28). Thus, transcription terminators within the 5′ region could help regulate expression of yeast genes. It is very probable that the 110-bp transcription terminator functions in some capacity as a terminator upstream of the *URA3* gene. The terminator described here could: (i) be directly involved in *URA3* regulation, (ii) terminate transcription of an unidentified upstream gene, or (iii) terminate transcription of a convergent and overlapping gene. The third possibility can probably be ruled out since Chevallier et al. (5) showed a greater than 99% asymmetry in the ability of separated *URA3* DNA strands to hybridize to mRNA. The terminator segment could be involved in the termination of an upstream gene since a genomic deletion of the *Hin*dIII *URA3* fragment (the upstream *Hin*dIII site was at base 1, Fig. 6) is a lethal event (Mark Rose, personal communication).

The first two possibilities are not mutually exclusive. It is possible that portions of some yeast promoters have the capacity to function as transcription terminators due to their inherent structures. Conversely, the presence of transcription terminators near to yeast promoters (or UAS structures) could enhance promoter activity. Thus, the physiological relevance for the observed *URA3* 5′ terminator activity could be to both terminate the transcription of an upstream gene and to enhance *URA3* transcriptional activity.

The *GAL7* promoter demonstrates regulatory features that could be analagous to *URA3*. The *GAL7* upstream activation site (UAS) is comprised of three elements (29). The two elements most proximal to the *GAL7* transcriptional start site (UAS1 and UAS2) have a similar dyad repeat structure and are the protected regions in *GAL4* binding experiments (3). In constrast, UAS3 shows no such dyad structure and is not protected in *GAL4* binding experiments. However, UAS3 does contain the sequence for a *CYC1*-like transcrip-

tion terminator as defined by Sherman (J. G. Yarger, M. C. Gorman, and G. Armilei, manuscript in preparation). The presence of UAS1 and UAS2 is sufficient for 75% of the normal induced *GAL7* activity. However, the presence of UAS3 increases total *GAL7* activity by 1/3 (29).

How might this be analagous to the *URA3* promoter? During the regulation of *URA3* transcription, the *PPR1* gene product acts to stimulate transcription of *URA1* and *URA3* in response to inducer accumulation (13). The *PPR1* effect has been shown to be exerted via the 5′ flanking region of the *URA3* gene (23). More recently, deletion analysis has suggested that the sequences essential for *PPR1* induction of *URA1* and *URA3* are located between the *TATA* box and the translation start codon (14). Within this region, *URA1* and *URA3* contain a common conserved sequence downstream of the TATA box, an extensive Pu:Py region including the TATA box, and an *upstream* conserved element (14). Thus, it is possible that the capacity of this *URA3* region to function as a transcription terminator could be due to the efficient binding of *PPR1* (or another *URA3* regulatory protein) to its upstream region. In contrast, a bonafide terminator element could serve to further enhance the effects of the *PPR1* gene product upon *URA3* transcription. We are now trying to differentiate between these possibilities.

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