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Termination Region in rRNA Genes from a Eucaryotic Thermophile, *Thermomyces lanuginosus*

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S1 mapping of the termination region in the ribosomal DNA from a thermophilic fungus, *Thermomyces lanuginosus*, revealed three distinct termini corresponding to the mature 25S rRNA, a precursor that is 19 nucleotides longer and corresponds to the 37S precursor in yeast cells, and a putative termination site at +96 that bears a limited sequence homology with the *SalI* box of mammalian cells. An estimate of the secondary structure suggested that the three termini are in close proximity, a feature that may be essential to precursor termination and maturation. The results raise questions regarding recently reported relationships between ribosomal DNA termination and spacer enhancer elements in fungi.

It is generally believed that signals for the termination of transcription are highly conserved in the course of evolution both with respect to different RNA polymerase gene families and also among widely divergent species (for reviews, see references 17 and 18). In procaryotes, the consensus signal includes a region of dyad symmetry (i.e., potential hairpin stem) preceded by a G+C-rich region and followed by a cluster of T residues on the noncoding (RNA-like) DNA strand (5, 18). Indeed, many eucaryotic genes that code for rRNA include at least some of these features. In *Xenopus* sp., for example, termination of the 5S rRNA genes occurs within clusters of four or more consecutive T residues (3); similar but longer clusters have been found to terminate the polymerase III transcription of 5S rRNA genes in yeast cells (12, 20) and have been observed in the ribosomal DNAs (rDNAs) from these same organisms (7, 20). Despite these similarities, considerable confusion remains with respect to both the great variations in the T-residue clusters and their relationship to adjacent sequences, which often contain a dyad symmetry. For example, in the yeast rDNA, Veldman and co-workers (21) pointed out an extensive dyad symmetry downstream of the putative termination site for RNA polymerase I which they speculated could play a role in the regulation of transcription termination. More recently, however, deletion analyses in the same laboratory of artificial rRNA minigenes suggest that a somewhat inefficient terminator is situated much further downstream at position +210, a T-residue cluster that appears to be associated with an enhancer sequence within the rDNA spacer (6). In the mouse genome, Kominami et al. (8) have noted a short T-residue cluster within a sequence that contains dyad symmetry, but again, more recently, Bartsch and coworkers (1) have shown that a tandemly repeated sequence distal to the termination site binds a termination protein factor that can terminate RNA polymerase I transcription.

Because thermophilic organisms contain significantly elevated G+C compositions, conserved A- or T-residue-rich areas of nucleotide sequence are often functionally important. Previously, we took advantage of these features in studies on the higher-order structure of the 5S and 5.8S rRNAs from a thermophilic fungus, *Thermomyces lanuginosus* (14, 22). In this study, we examined the rRNA genes of this same thermophile for sequence elements that are important in termination and for structural features that play a role in rRNA maturation. The sequence for the termination region was determined from *Hin*fI, *Hpa*II, or *Taq*I restriction endonuclease digestion fragments of a larger *Eco*RI fragment which were isolated from a previously constructed (15) λ Charon 4A vector (2) containing the complete rDNA unit and subcloned (11) by using the pBR322 plasmid vector (pTL509) (Fig. 1). Longer sequences were determined more than once by sequencing overlapping portions of both strands of the same fragment; the termination sequence was further confirmed when the opposite strand was labeled and sequenced during S1 mapping. The mature 25S rRNA sequence was highly homologous to sequences previously reported for other fungal rRNAs.

When the termination region in *T. lanuginosus* (Fig. 2) is compared with those of other fungi, the T-residue clusters that have been alluded to in other yeast rDNAs are clearly not present. Unlike the case for *Saccharomyces carlsbergensis* (21), no extremely T-rich region was observed close to the 3′ end of the 25S rRNA sequence, and unlike *Saccharomyces cerevisiae* (20), no T-rich cluster was observed about 220 base pairs downstream. Veldman and co-workers (21) initially suggested that an extensive dyad symmetry immediately downstream of the extremely T-rich

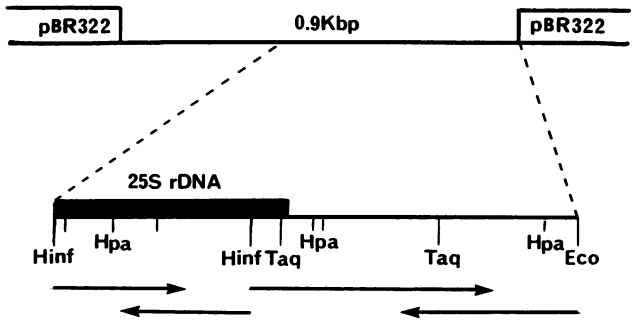

FIG. 1. Determination of the nucleotide sequence for the rDNA termination region from *T. lanuginosus*. A subcloned *Eco*RI cleavage fragment (pTL509) that contained the termination region (15) was further cleaved with restriction endonuclease *Hin*fI, *Hpa*II, or *Taq*I and labeled; sequences were determined by using the chemical degradation technique of Maxam and Gilbert (12). Restriction sites relevant to the termination region are as indicated; arrows show the direction and extent of each sequence obtained.

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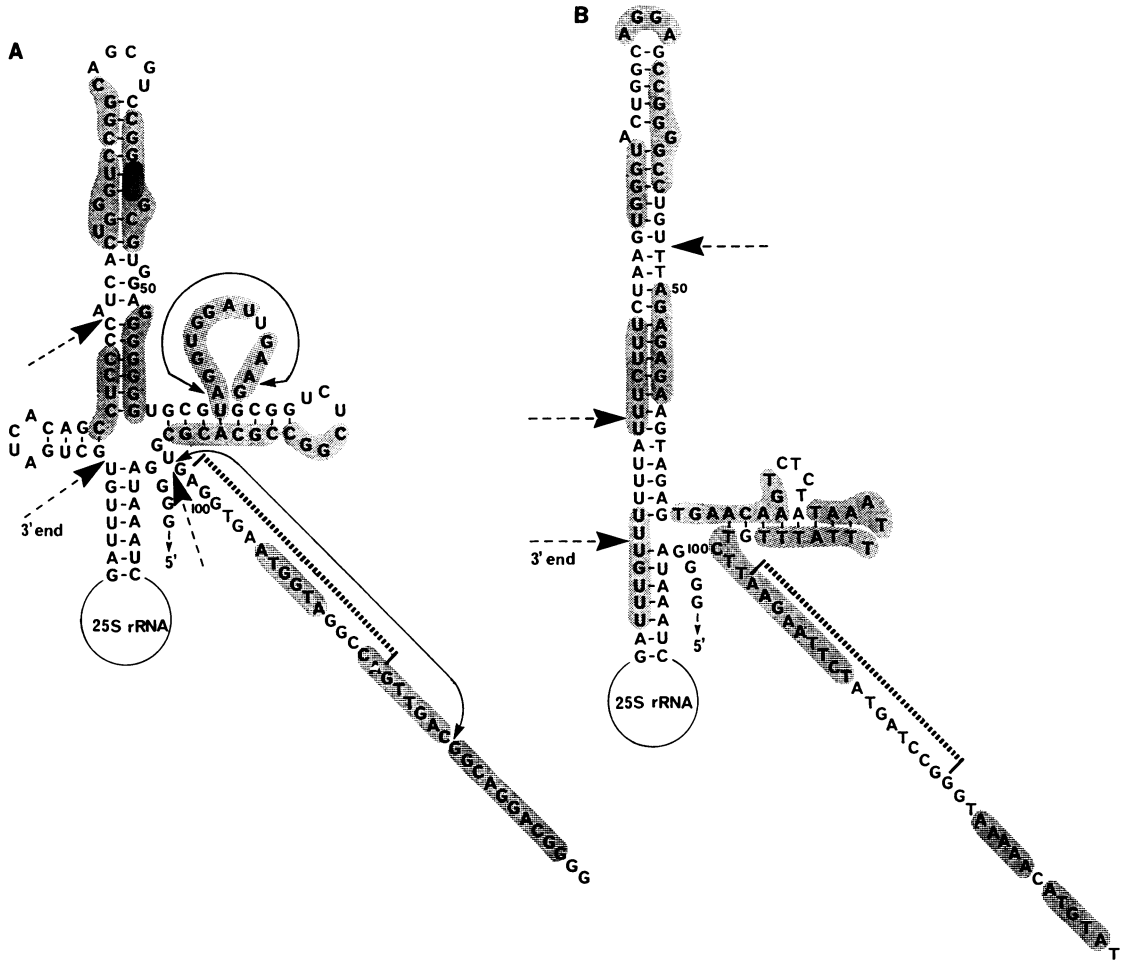

FIG. 2. Comparison of the termination regions for rDNA transcripts from *T. lanuginosus* (A) and *S. cerevisiae* (B). The secondary structures are computer-aided estimates that maximize the numbers of base pairs (19). Broken arrows indicate the mature or precursor rRNA termini; small arrows enclose cleavage sites resulting from S1 nuclease mapping in the absence of homologous RNA. Shaded areas indicate symmetrical sequence blocks; thick broken lines enclose *Sal*I box-like sequences. Uridylate residues (U) indicate regions for which transcripts have been demonstrated by S1 mapping; thymidylate residues (T) indicate the distal DNA sequence.

region that follows the gene, together with the T-rich region, were important for termination. Indeed, they used hybridization experiments and sequence analysis to establish that a 37S precursor rRNA terminated in the T-rich cluster just seven nucleotides beyond the 3′ end of the 25S rRNA but have now concluded that these features probably function in the processing of nascent RNA (6). In *T. lanuginosus*, even the proximal T-rich region was absent and only the extensive dyad symmetry (hairpin structure) was present (Fig. 2).

Because the thermophilic sequence was very different, the actual termination sites of precursors were sought by S1 nuclease mapping. We anticipated that the thermophilic nature of this organism might make precursor molecules more stable to purification and therefore termination and precursor sites more apparent. When the hybrids were digested with S1 nuclease over a wide range of enzyme concentrations, cleavages were consistent at three distinct sites (Fig. 3). The major cleavage site corresponded to the 3′ end of the 25S rRNA sequence, and additional cleavage sites were centered downstream, at +19 and +96 (Fig. 2). Since these cleavages were not observed in the absence of complementary RNA (Fig. 4), they clearly represent the termini of extended RNA precursors. Neither of the extended sequences was consistent with a cluster of T acid residues being essential in the termination.

Estimates of the secondary structure for the extended regions were examined to gain an understanding of the two extended cleavage sites. Although the nucleotide sequences were clearly not homologous, as recently noted in several other fungi (6), an analogous extensive dyad symmetry was present in both the yeast and thermophilic fungus sequences (Fig. 2). In contrast to the same report, however, the conserved sequence elements that were noted by Kempers-Veenstra and co-workers (6) in several other fungi are not present in *T. lanuginosus*, i.e., neither the C-U-A-A/U-U-A-G-A block in the stem nor the G-C-A-G-G-A-G-G-C terminal loop. In the thermophile, the first of the two extended sites appears likely to be a processing site that gives rise to an intermediate rRNA precursor, equivalent to the 37S precursor rRNA reported by Veldman and co-workers (21). Since longer precursors were not observed (Fig. 3), the second site at T +96 appears to be the actual termination site. It contains no T-rich cluster but seems folded into an extensive secondary structure that brings the site into close proximity with

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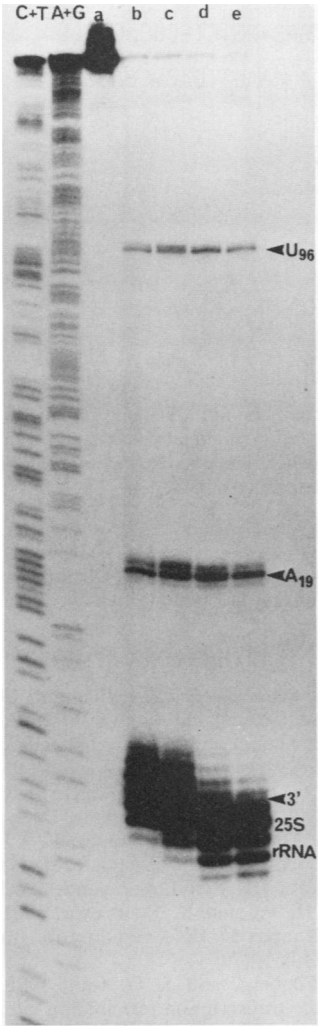

FIG. 3. S1 nuclease mapping of rRNA precursors in *T. lanuginosus*. Mycelial whole-cell RNA was prepared by sodium dodecyl sulfate-phenol extraction (10), and a 355-base-pair DNA fragment of *Hin*fI-*Eco*RI-digested pTL509 containing the downstream region (Fig. 1) was labeled at the 3′ end of the coding strand with [α-^{32}P]dCTP and Klenow enzyme (11) and gel purified after strand separation (13). RNA (20 μg) was incubated with labeled DNA at 42°C for 16 h in 50 μl of 50% formamide in 0.4 M sodium chloride–10 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES; pH 6.4) and rapidly chilled on ice. Hybrids were digested at 25°C for 4 h with 0 (lane a), 50 (lane b), 100 (lane c), 200 (lane d), or 400 (lane e) U of S1 nuclease in 15 mM zinc sulfate–2 mM sodium chloride–30 mM sodium acetate (pH 4.5; total volume, 400 μl), extracted with phenol-chloroform, and analyzed on a 12% polyacrylamide sequencing gel. 3′-End-labeled rDNA was also chemically degraded as described by Maxam and Gilbert (13), and digests were included (lanes C+T and A+G) as markers to determine the cleaved nucleotides.

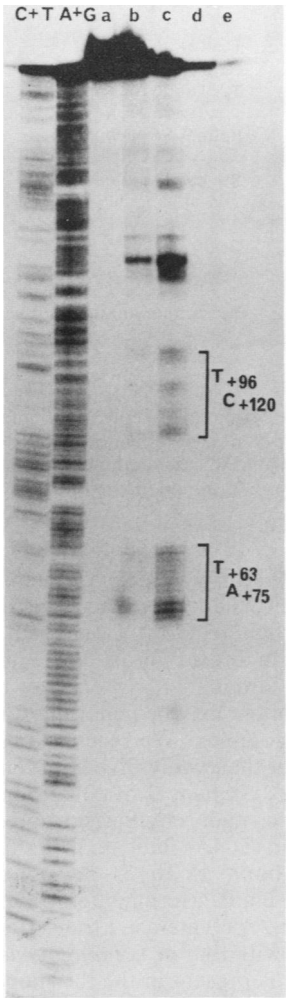

FIG. 4. S1 nuclease-sensitive sites in the coding strand from the termination regions of rDNA in *T. lanuginosus*. 3′-End-labeled coding strand plus whole-cell *Escherichia coli* nucleic acid carrier (total, 20 μg) was digested with 0 (lane a), 0.1 (lane b), 1.0 (lane c), 100 (lane d), or 400 (lane e) U of S1 nuclease, extracted with phenol-chloroform, and analyzed on a 12% polyacrylamide sequencing gels. Chemically degraded rDNA was included (lanes C+T and A+G) as markers to determine the cleaved nucleotides.

the 3′ end of the 25S rRNA sequence as well as the first extended cleavage site (+19). The nucleotide sequence that surrounds this site is not unusual with one exception: the termination region contains an unusually high number of symmetrical sequence blocks (Fig. 2).

Preliminary support for the secondary-structure estimate suggested by Fig. 2 was obtained when the single-stranded 3′-end-labeled rDNA that was used in S1 mapping was partially digested with low concentrations of S1 nuclease in the presence of nonhomologous carrier RNA. The initial sites of cleavage corresponded to the extended unpaired regions in the estimate (Fig. 4), residues T +63 to approximately G +73 and T +96 to about A +119 (enclosed by small arrows in Fig. 2).

Sequence comparisons also suggested that the features noted in the *T. lanuginosus* rDNA termination region may be important to precursor processing, transcription termination, or both. As illustrated with yeast rDNA (Fig. 2), our estimates of the secondary structure for the equivalent region in other rDNAs (not shown) retain similar features, i.e., an extensively folded region ending in an unpaired sequence and a series of symmetrical sequence blocks. Although there is no sequence conservation and the predicted secondary structure can differ significantly among organisms, we note that in each example for which the termini are known, the estimate of the secondary structure appears to fold all of the termini into a single structural domain (perhaps a processing domain). This could explain

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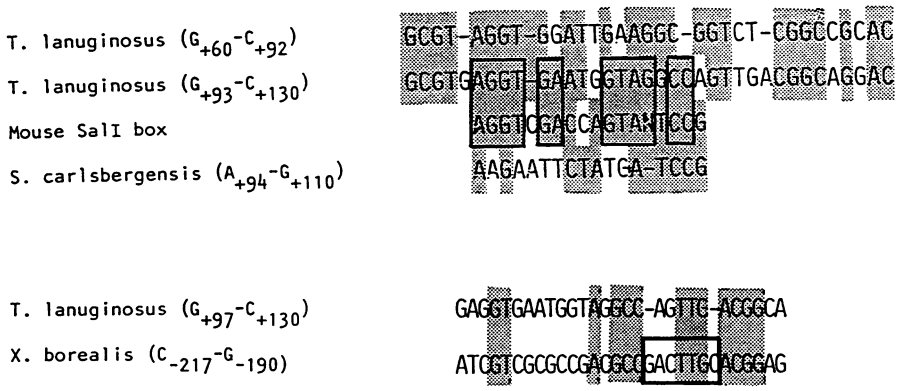

FIG. 5. Putative termination sequence elements in *T. lanuginosus* rDNA. The upper comparison indicates *SalI* box-like elements in the thermophile and a potentially equivalent sequence in *S. carlsbergensis* (Fig. 2); the mouse *SalI* box sequence is taken from Bartsch et al. (1). The lower comparison indicates homology with the termination sequence in *Xenopus borealis*. The enclosed element is highly homologous in all known *Xenopus* termination sites (9); both sequences immediately follow the S1-mapped terminus.

the recent observation by Kempers-Veenstra et al. (6) that deletion of sequences downstream of position +43 abolishes the formation of mature 25S RNA molecules and precursors ending at +10. In other words, the extensive secondary structure in the termination region may not function as a termination signal but may be important for the organization of a processing complex. We recently made a similar suggestion to explain the widely divergent lengths in the intervening transcribed sequences of eucaryotic rDNAs (16).

As noted earlier, studies by Bartsch and co-workers (1) on mouse and human rDNAs indicate that a tandemly repeated sequence (*SalI* box), 15 to 25 nucleotides distal to the termination site, binds a termination protein factor (4) that can terminate RNA polymerase I transcription. Comparison of this sequence with that in *T. lanuginosus* indicated that a similar sequence is present in the thermophile, immediately distal to the putative termination site (G +60 to C +92) (Fig. 5). Furthermore, a less homologous but related sequence immediately precedes it (G +93 to C +130), and an equivalent sequence may even be present in the yeast sequence (A +94 to G +110). Although the *SalI* box has not been identified in the *Xenopus* genome, it may also be important that when the *Xenopus* termination sites (9) are aligned with the putative *Thermomyces* termination site (Fig. 5), homology is evident in the region which was most conserved in the *Xenopus* sequence.

Finally, these results raise further questions about the relationship between rDNA spacer promoter or enhancer elements and termination signals that they appear to contain (1, 6, 9). Kempers-Veenstra and co-workers (6) have noted that in yeast cells, deletion of sequences that are required for formation of the 3′-ends in the 26S rRNA and 37S precursor molecules reveals a weak termination at position 210, a site that maps in an enhancing element for rDNA transcription. In some contrast, Bartsch and co-workers (1) also have noted a termination site in the upstream region of mouse ribosomal genes, but this site is distinct from the *SalI* box cluster that constitutes the primary termination site. Although nothing is yet known about the promoter and enhancer elements in *T. lanuginosus*, the results of this study appear to be similar to observations for the mammalian rDNA and raise the possibility that the deletion studies in yeast cells have masked an efficient but very rapidly processed termination site in these cells. Additional studies in other fungi may be helpful in clarifying this possibility.

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LITERATURE CITED

1. **Bartsch, I., C. Schoneberg, and I. Grummt.** 1987. Evolutionary changes of sequences and factors that direct transcription termination of human and mouse ribosomal genes. Mol. Cell. Biol. **7**:2521–2529.
2. **Blattner, F. R., B. G. Williams, A. E. Blechl, K. Denniston-Thompson, H. E. Faber, L. A. Fiulong, D. J. Grunwald, D. O. Kiefer, D. D. Moore, J. W. Schumm, E. L. Sheldon, and O. Smithies.** 1977. Charon phages: a safer derivative of bacteriophage lambda for DNA cloning. Science **196**:161–169.
3. **Bogenhagen, D. F., and D. D. Brown.** 1981. Nucleotide sequences in Xenopus 5S DNA required for transcription termination. Cell **24**:261–270.
4. **Din, N., J. Engberg, and J. G. Gall.** 1981. The nucleotide sequence at the transcription termination site of the ribosomal RNA gene in *Tetrahymena thermophila*. Nucleic Acids Res. **10**:1503–1513.
5. **Gerbi, S. A.** 1985. Evolution of ribosomal DNA, p. 419–517. *In* R. J. MacIntyre (ed.), Molecular evolutionary genetics. Plenum Publishing Corp., New York.
6. **Kempers-Veenstra, A. E., J. Oliemans, H. Offenberg, A. F. Dekker, P. W. Piper, R. J. Planta, and J. Klootwijk.** 1986. 3′-End formation of transcripts from the yeast rRNA operon. EMBO J. **5**:2703–2710.
7. **Klootwijk, J., P. de Jonge, and R. J. Planta.** 1979. The primary transcript of the ribosomal repeating unit in yeast. Nucleic Acids Res. **6**:27–39.
8. **Kominami, R., Y. Mishima, Y. Urano, M. Sakai, and M. Muramatsu.** 1982. Cloning and determination of the transcription termination site of ribosomal RNA gene of the mouse. Nucleic Acids Res. **10**:1963–1979.
9. **Labhart, P., and R. H. Reeder.** 1986. Characterization of three sites of RNA 3′ end formation in the Xenopus ribosomal gene spacer. Cell **45**:431–443.
10. **Lucas, M. C., J. W. Jacobson, and N. H. Giles.** 1977. Characterization and in vitro translation of polyadenylated messenger ribonucleic acid from *Neurospora crassa*. J. Bacteriol. **130**: 1192–1198.
11. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Maxam, A. M., and W. Gilbert.** 1977. A new method for sequencing DNA. Proc. Natl. Acad. Sci. USA **74**:560–564.
13. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–559.

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14. **Nazar, R. N., and A. T. Matheson.** 1977. Nucleotide sequence of *Thermus aquaticus* ribosomal 5S ribonucleic acid. J. Biol. Chem. **252**:4256–4261.
15. **Nazar, R. N., and W. M. Wong.** 1985. Is the 5S RNA a primitive ribosomal RNA sequence? Proc. Natl. Acad. Sci. USA **82**: 5608–5611.
16. **Nazar, R. N., W. M. Wong, and J. L. A. Abrahamson.** 1987. Nucleotide sequence of the 18-25S ribosomal RNA intergenic region from a thermophile, *Thermomyces lanuginosus*. J. Biol. Chem. **262**:7523–7527.
17. **Platt, T.** 1986. Transcription termination and the regulation of gene expression. Annu. Rev. Biochem. **55**:339–379.
18. **Rosenberg, M., and D. Court.** 1979. Regulatory sequences involved in the promotion and termination of RNA transcription. Annu. Rev. Genet. **13**:319–353.
19. **Tinoco, I., Jr., P. N. Borei, B. Dengler, M. D. Levine, O. C. Uhlenbeck, D. M. Crothers, and J. Gralla.** 1973. Improved estimation of secondary structure in ribonucleic acids. Nature (London) New Biol. **246**:40–41.
20. **Valenzuela, P., G. I. Bell, A. Venegas, E. T. Sewell, F. R. Masiarz, L. J. DeGennaro, F. Weinberg, and W. J. Rutter.** 1977. Ribosomal RNA genes of *Saccharomyces cerevisiae*. II. Physical map and nucleotide sequence of the 5S ribosomal gene and adjacent intergenic regions. J. Biol. Chem. **252**:8126–8135.
21. **Veldman, G. M., J. Klootwijk, P. de Jonge, R. J. Leer, and R. J. Planta.** 1980. The transcription termination site of the ribosomal RNA operon in yeast. Nucleic Acids Res. **8**:5179–5192.
22. **Wildeman, A. G., and R. N. Nazar.** 1981. Studies on the secondary structure of 5.8S RNA from a thermophile, *Thermomyces lanuginosus*. J. Biol. Chem. **256**:5675–5682.

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