5′- and 3′-Terminal Nucleotide Sequences of *Tetrahymena pyriformis* 17S rRNA†

Edward G. Niles

ABSTRACT: Ribonuclease T_1 oligonucleotides arising from the 5′ and 3′ termini of the 17S rRNA of *Tetrahymena pyriformis* were isolated by the diagonal method of Dahlberg (Dahlberg, J. E. (1968), *Nature (London) 220,* 548), and their nucleotide sequences were determined. The base sequence of the 3′-terminal fragment is (G)AUCAUUA$_{OH}$, which is identical to that found in other 17S–18S eucaryotic rRNA species. The nucleotide sequence of the 5′-terminal oligonucleotide is pAACCUGp, which is identical in length to that found in other eucaryotes and shows a partial but significant sequence homology to the 5′ RNase T1 oligonucleotides isolated from other eucaryotic species.

The nucleotide sequences of the terminal regions of an RNA molecule are of interest for several reasons. First, in a primary transcript, these sequences are derived from a portion of the regions of the DNA responsible for initiation and termination of transcription. Second, if the RNA is a product of a processing event, information about the substrate base sequence or structural specificity may be deduced. Third, as in the case of the 3′ terminal region of the 16S rRNA of *Escherichia coli,* a function may be ascribed to the terminal oligonucleotide.

It has recently been suggested that the 3′-terminal region of the 16S rRNA of *E. coli* is involved in the initiation of translation by base pairing to a sequence in the mRNA 5′ to the initiation codon (Shine and Dalgarno, 1974b). It has been demonstrated that this interaction does take place at the bacteriophage R17 A protein ribosome-binding site, in vitro (Steitz and Jakes, 1975). The generalization of the interaction between the 3′-terminal portion of the 17S to 18S rRNA of eucaryotes and mRNA has been made on the basis that an identical octanucleotide is found at the 3′ end of each eucaryotic 17S–18S rRNA species, (G)AUCAUUA$_{OH}$ (Hunt, 1970; Dalgarno and Shine, 1973; Shine and Dalgarno, 1974a; Eladari and Galibert, 1976; Sprague et al., 1975). Furthermore, this sequence is partially complementary to a region of the ribosome binding site of some eucaryotic mRNAs (Dasgupta et al., 1975; Legon, 1976).

There is evidence that the 5′-terminal sequence of eucaryotic 17S–18S rRNA shows partial sequence homology (Sakuma et al., 1976; Eladari and Galibert, 1975). However, this hexanucleotide sequence has not been as stringently conserved, since the sequence of the 5′ end of the 18S rRNA from the silk worm *Bombyx mori* is identical only in the first, third, and sixth bases (Sprague et al., 1975).

As part of an on going investigation of the synthesis and processing of the ribosomal RNA precursor molecule in the ciliated protozoan, *Tetrahymena pyriformis,* the nucleotide sequences of the 5′- and 3′-terminal oligonucleotides of the 17S rRNA were determined. As found in other eucaryotes, the base sequence of the 3′ end, (G)AUCAUUA$_{OH}$, is conserved. This lends further credence to the universality of a role of this region of the 17S–18S rRNA in protein synthesis. The nucleotide sequence of the 5′ end is pAACCUGp which shares a five-base homology with the mouse 18S rRNA (Eladari and Galibert, 1975) and a three or five base homology with the partial sequence reported for six other eucaryotes (Sakuma et al., 1976), but only a two-base homology with the 5′ end of the 18S rRNA from *Bombyx mori* (Sprague et al., 1975).

Materials and Methods

RNase$^+$ T_1, T_2, and U_2 were obtained from Calbiochem; snake venom phosphodiesterase, pancreatic RNase, and pancreatic DNase$^+$ (RNase free) were purchased from Worthington; $H_3{}^{32}PO_4$ was purchased from New England Nuclear Corp.; and bacterial alkaline phosphatase was isolated from *E. coli* by the procedure of Torriani (1966).

Cell Growth. The amicronucleate strain of *Tetrahymena pyriformis,* GL, was obtained from Dr. K. Karrer. The organism was grown and maintained as described by Gorovsky et al. (1975).

Labeling Conditions and rRNA Isolation. rRNA was labeled to a specific activity of 10^5 to 5×10^5 cpm/μg by growing *T. pyriformis* overnight to a density of $5–8 \times 10^5$ cells/mL in 50 mL of a medium containing 2% proteose peptone, 0.1% yeast extract, and 2–10 mCi of $H_3{}^{32}PO_4$. The cells were collected by centrifugation at 2000*g* for 5 min. The cell pellet was solubilized by resuspension in 4 mL of a lysis solution containing 0.1 M Tris-Cl,[1] pH 8.0, 0.5% sodium dodecyl sulfate, and 40 mM EDTA. The lysis step and all subsequent procedures were carried out on ice in order to prevent the melting of the 25S rRNA which yields two breakdown products of about 16S in size (Bostock et al., 1971). The lysate was extracted twice with a solution of phenol–chloroform–isoamyl alcohol (50:50:1) and the nucleic acid was precipitated from the aqueous layer by the addition of 2 volumes of 95% ethanol.

The nucleic acid precipitate was collected by centrifugation at 10 000*g* and resuspended in 1 mL of 0.1 M Tris-Cl, pH 8, 1 mM EDTA. After the solution had been adjusted to 5 mM $MgCl_2$, the DNA was removed by digestion with pancreatic DNase, 25 μg/mL for 15 min at 4 °C. The RNA components

† From the Department of Biochemistry, School of Medicine, State University of New York at Buffalo, Buffalo, New York 14214. *Received December 2, 1976.* This work was supported by Public Health Service Grant GM 23259 and General Research Funds of the State University of New York at Buffalo.

[1] Abbreviations used are: EDTA, ethylenediaminetetraacetic acid; RNase, ribonuclease; DNase, deoxyribonuclease; Tris-Cl, 2-amino-2-hydroxymethyl-1,3-propanediol chloride; DEAE, diethylaminoethyl; EDTA, (ethylenedinitrilo)tetraacetic acid.

were separated by centrifugation in a 15–30% sucrose gradient in an SW 27 rotor for 20 hr at 25 000 rpm, 4 °C (McConkey, 1967). The 17S rRNA-containing fractions were pooled and the 17S rRNA was precipitated by the addition of 2 volumes of 95% ethanol.

Isolation of the 5′- and 3′-Terminal Oligonucleotides. The 17S rRNA was collected by centrifugation at 10 000 rpm and 100–200 μg of 17S rRNA was taken up in 10 μL of an RNase T_1 solution, 1 mg/mL, in 0.01 M Tris-Cl, pH 7.5, 1 mM EDTA, (Barrell, 1971), and digestion was carried out for 1 h at 37 °C. Cyclic phosphate digestion intermediates were removed by adjusting the digestion mixture to 0.1 N HCl and further incubating at 37 °C for 30 min (Dahlberg, 1968). The 5′- and 3′-termini-containing oligonucleotides were separated from the internal oligonucleotides by the diagonal method of Dahlberg (1968). Electrophoresis in the first dimension was carried out for 20 h at 14 V/cm and in the second dimension for 8 h at 18 V/cm.

The oligonucleotide which migrates ahead of the internal oligonucleotides in the second dimension was demonstrated to contain the 5′ terminus by pretreating 17S rRNA in 0.5 mL of bacterial alkaline phosphatase, 100 μg/mL, in 0.1 M Tris-Cl, pH 8.0. After incubating for 1 h at 37 °C, the 17S rRNA was reisolated by extraction of the incubation mixture with phenol and precipitation with 2 volumes of 95% ethanol. The alkaline phosphatase pretreated 17S rRNA was digested with RNAse T_1 and the products were separated by the diagonal method as described above.

Base Sequence Determination. The nucleotide sequences of the isolated terminal oligonucleotides were carried out by standard techniques (Barrell, 1971).

Results

Isolation of the Terminal Oligonucleotides. The diagonal method of Dahlberg (1968) permits one to identify the 5′- and 3′-termini-containing oligonucleotides by virtue of a shift in the mobility of an oligonucleotide after the removal of its external phosphates. The products of the RNase T_1 digestion are separated by electrophoresis on DEAE-cellulose in 7% formic acid, in large part by the number of phosphates that they contain; the larger oligonucleotides, containing more phosphates, migrate more slowly. After electrophoresis in one dimension, the strip of paper is treated with bacterial alkaline phosphatase which hydrolyzes all external phosphates. The strip is then sewn onto another sheet of DEAE-cellulose paper and electrophoresis is carried out perpendicular to the first dimension. Alkaline phosphatase removes one phosphate from the internal oligonucleotides and as a result they gain in mobility in the second dimension in proportion to their size. The 3′-termini-containing oligonucleotide loses no external phosphate, so it does not gain in mobility in the second dimension. Subsequently, it can be identified because it migrates more slowly in the second dimension than do the internal oligonucleotides. The 5′-end-containing oligonucloetide loses from two to four external phosphates after alkaline phosphatase treatment, greatly increasing its mobility in the second dimension, and thus can be identified by migrating ahead of the internal oligonucleotides.

Figure 1 is a photograph of the two-dimensional separation of the T_1 oligonucleotides of *T. pyriformis* 17S rRNA. The 5′- and 3′-end-containing oligonucleotides are indicated by the arrows. The yields of the ends were 60 and 90%, respectively, in this preparation and range from 40 to 90% in different isolations.

In order to demonstrate that the spot which migrates ahead of the internal oligonucleotides in the second dimension contains the 5′ terminus, intact 17S rRNA was pretreated with bacterial alkaline phosphatase to remove any external phosphates. The pretreated 17S rRNA was reisolated by phenol extraction and, after RNase T_1 digestion, the oligonucleotides were separated by the diagonal method (Dahlberg, 1968). The alkaline phosphatase pretreated rRNA lacked any trace of the apparent 5′-terminal oligonucleotide but contained normal amounts of the 3′-terminal oligonucleotide (70% yield). This demonstrates that the apparent 5′-terminal oligonucleotide which migrates ahead of the internal oligonucleotides in the second dimension (Figure 1) must contain at least one 5′-phosphate prior to alkaline phosphatase treatment and must, therefore, originate at the 5′ end of the intact 17S rRNA.

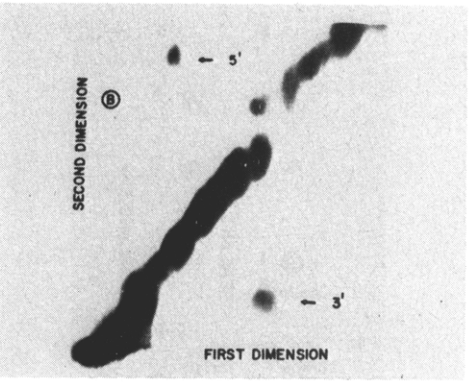

FIGURE 1: Location of the 5′- and 3′-terminal oligonucleotides by the diagonal method (Dahlberg, 1968). *T. pyriformis* 17S rRNA, digested with RNase T_1 and treated with 0.1 N HCl to remove cyclic phosphate digestion intermediates, was electrophoresed on DEAE-cellulose in 7% formic acid for 20 h at 14 V/cm. The strip of paper containing radioactivity was cut out and the external phosphates were removed by treatment with bacterial alkaline phosphatase. The reaction was terminated by washing the paper in 95% ethanol and the strip was sewn into a second sheet of DEAE-cellulose and electrophoresis was carried out perpendicular to the first dimension in 7% formic acid for 8 h at 18 V/cm. Positions of the 5′- and 3′-terminal oligonucleotides are indicated.

Further evidence in support of the contention that the apparent 3′ terminal oligonucleotide comes from the 3′ end of the 17S rRNA is presented in Table IV. Total digestion of this oligonucleotide with snake venom phosphodiesterase does not produce a pG residue; however, products of RNase T_1 digestion have G as the 3′ nucleotide. Therefore, since this oligonucleotide lacks G at its 3′ end and is isolated in high yield (90%), it is likely to have arisen from the 3′ end of the 17S rRNA.

Base Sequence of the 3′-Terminal Oligonucleotide. The nucleotide sequence of the 3′-end-containing oligonucleotide was determined by standard techniques (Barrell, 1971). Treatment with pancreatic RNase, specific for pyrimidines, yields three products, Up, Cp, and ApUp, in a molar ratio of 1:1:2 (Figure 2 and Table I). Digestion with RNase U_2, specific for A residues in RNase T_1 products, yields Ap, compound B, and compound C (Figure 3 and Table II). The sequence of compound B was determined to be $UpUpA_{OH}$ by further digestion of equal amounts of compound B by snake venom phosphodiesterase or RNase T_2 which yields equal molar amounts of pU and pA, and 2 mol of Up, respectively (Table III). The base sequence of compound C was determined by further digestion of compound C with venom phosphodiesterase and RNase T_2 which yields equal molar amounts of pAp and pC, and 1 mol each of Up, Cp, and Ap, respectively (Table III). The sequence of compound C must, therefore, be UpC-pAp. The three RNase U_2 products were ordered by taking into

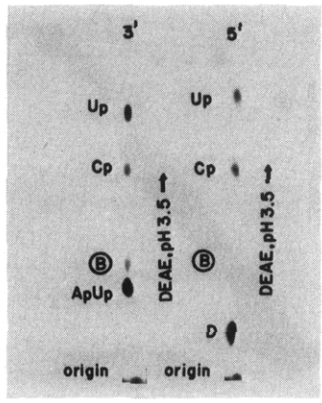

FIGURE 2: Separation of the pancreatic RNase digestion products of the isolated 5′- and 3′-terminal oligonucleotides. The isolated oligonucleotides were taken up in 10 μL of pancreatic RNase (0.1 mg/mL), 0.01 M Tris-Cl, pH 7.5, 1 mM EDTA (Barrell, 1971) and incubated at 37 °C for 30 min. The products were separated by electrophoresis on DEAE-cellulose at pH 3.5 for 1 h at 40 V/cm.

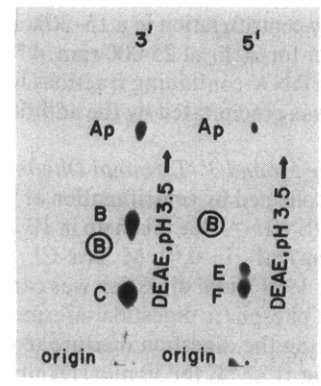

FIGURE 3: Separation of the RNase U_2 digestion products of the isolated 5′- and 3′-terminal oligonucleotides. The 5′- and 3′-terminal oligonucleotides were taken up in 10 μL of 0.1 units/mL RNase U_2 in 0.05 M sodium acetate, 2 mM EDTA, 0.1 mg/mL bovine serum albumin (Barrell, 1971), and, after digestion for 2 h at 37 °C, the products were separated by electrophoresis for 1 h at 40 V/cm on DEAE-cellulose.

TABLE I: Products Obtained by Pancreatic RNase Digestion of the 5′- and 3′-Terminal Oligonucleotides.

3′	Mole ratio	5′	Mole ratio
Up	1	Up	0.9
Cp	1.1	Cp	1.0
ApUp	1.7	Compound D	1.1

TABLE II: Products Obtained by RNase U_2 Digestion of the 5′- and 3′-Terminal Oligonucleotides.

3′	Mole ratio	5′	Mole ratio
Ap	1.0	Ap	0.5
Compound B	1.0	Compound E	1.3
Compound C	1.0	Compound F	1.0

TABLE III: Mole Ratios of the Products Obtained by Further Enzymatic Hydrolysis of Compounds B to F by Digestion with RNase T_2 or Snake Venom Phosphodiesterase (V).

	Compd B		Compd C		Compd D		Compd E		Compd F	
	T_2	V	T_2	V	T_2	V	T_2	V	T_2	V
U	2	1	1						1	1
C			1	1	1				2	1
A		1	1.1		2.2		2.1			
G										1
pAp				1.2				1.0		

account the products of pancreatic RNase which yields 2 mol of ApUp. This defines the sequence as being: (G)ApUpCpA-pUpUpA$_{OH}$.

Sequence Analysis of the 5′-Terminal Oligonucleotide. Treatment of the 5′-end-containing oligonucleotide with pancreatic ribonuclease yields three products: Up, Cp, and compound D (Figure 2 and Table I). The base sequence of compound D was determined to be ApApCp by complete digestion with RNase T_2 which yields 2 mol of Ap to 1 mol of Cp (Table III). Hydrolysis of the 5′ oligonucleotide by RNase U_2 yields three products, Ap, compound E, and compound F (Figure 3, Table II). The sequence of compound E was determined to be ApAp by further digestion with snake venom phosphodiesterase or RNase T_2 which yields 1 mol of pAp and 2 mol of Ap, respectively (Table III). It is known that under these conditions RNase U_2 digests ApA bonds slowly, resulting in the production of $(Ap)_N$ partial products (Barrell, 1971). Compound F upon digestion with RNase T_2 yields 2 mol of Cp to 1 mol of Up (Table III). Venom phosphodiesterase digestion yields 1 mol of pU, pC, and pG (Table III). The partial order of compound E is thus C(Up,Cp)G and the order of the U_2 products must be ApApCp(Cp,Up)G. Since a 5′ purine is unusual in a rRNA-processing product, the presence of the 5′-A residue was confirmed by comparing the base composition resulting from total hydrolysis catalyzed by either RNase T_2 or venom phosphodiesterase (Table IV). Venom digestion yields 1 mol of pG as would be expected for a RNase T_1 product, and also contains 1 mol less of A than do the T_2 digestion products (Table IV). This confirms the presence of A at the 5′ end of the 17S rRNA.

TABLE IV: Mole Ratios of the Products Obtained by Total Digestion of the 5′- and 3′-Terminal Oligonucleotides either by RNase T_2 or by Snake Venom Phosphodiesterase (V).

	5′		3′	
	T_2	V	T_2	V
U	1.0	1.1	3.0	3.0
G		1.0		
A	2.0	1.1	2.0	2.0
C	1.9	2.0	1.2	1.2

The ambiquity in the placement of the Up and Cp residues in positions 4 and 5 was resolved by characterizing the pancreatic RNase digestion products subsequent to modification of the U and G residues by CMC^+ (Barrell, 1971). Digestion with pancreatic RNase yields 1 mol of ApApCp, 1 mol of Cp, and 0.3 mol of U*pG*. The yield of U*pG* is low and is probably due to a partial modification of these residues. A significant amount of radioactivity can be found streaking toward the cathode. In spite of the low yield of U*pG*, the presence of equal molar amounts of ApApCp and Cp remove any ambiquity. Thus, the sequence of the 5′-terminal oligonucleotide is pApApCpCpUpGp.

Discussion

In this report is presented the base sequence of the terminal oligonucleotides isolated from RNase T_1 digestion of the 17S

TABLE V: A Comparison of the Hexanucleotide Sequences Obtained from the 5′ Ends of Several Eucaryotic 17S–18S rRNAs.

pApApCpCpUpGp	*Tetrahymena pyriformis*
pUpApCpCpUpGp	Mouse (Eladari and Galibert, 1975)
pUpApCp(Cp,Up)Gp	Hela cells (Sakuma et al., 1976)
	Rat
	Chicken
	Xenopus laevis
	Sea urchin
	Dictyostelium discoideum
pUpUpCpUpCpGp	*Bombyx mori* (Sprague et al., 1975)

rRNA of *Tetrahymena pyriformis*. The sequence (G)AUCAUUA$_{OH}$ is found at the 3′ end and is identical to that found in other eucaryotic 17S–18S rRNA molecules (Hunt, 1970; Dalgarno and Shine, 1973; Shine and Dalgarno, 1974a; Eladari and Galibert, 1975; Sprague et al., 1975). The conservation of this sequence in all eucaryotic species argues for a functional role of this region of the 17S–18S rRNA. The complementarity of this sequence to a portion of the ribosome-protected region of some eucaryotic mRNAs (Dasgupta et al., 1975; Legon, 1976) suggests that the 17S–18S rRNA and mRNA may interact in protein synthesis in eucaryotes as they do in *E. coli* (Shine and Dalgarno, 1974b; Steitz and Jakes, 1975).

In the case of the 5′-terminal oligonucleotide there is only partial but significant sequence homology (Table V). In their partial sequence analysis of the 5′ ends of 18S rRNA from seven eucaryotic species, Sakuma et al. (1976) demonstrated that the sequence pUAC(C,U)G predominates. This is identical to the sequence of the mouse 18S rRNA, pUACCUG, characterized by Eladari and Galibert (1976), but shows only a limited three-base homology to that of the 5′ end of 18S rRNA from *Bombyx mori*, pUCCUCG (Table V) (Sprague et al., 1975).

The sequence of the 5′ end of the *T. pyriformis* 17S rRNA, pAACCUGp, shows a five-base homology with the mouse 18S rRNA (Eladari and Galibert, 1975), and a three- to five-base homology (depending upon the placement of Cp and Up in positions 4 and 5) with the partial sequences reported for six other eucaryotic species (Sakuma et al., 1976). However, a comparison of the *Tetrahymena pyriformis* sequence to that of *Bombyx mori* reveals only a two-base homology (Table V).

In summary, the 5′-terminal hexanucleotide of eucaryotic 17S–18S rRNA shows a partial but significant sequence homology. In eight of nine cases, there is a three- to five-base conservation of the six-base sequence (Table V). The species showing the lowest degree of homology, *Bombyx mori*, still has at least a ⅗ base conservation with the most common sequence. One must now consider a role for the 5′ end of the 17S to 18S rRNA in one of several functions, i.e., rRNA processing or ribosome assembly.

Acknowledgments

I express my gratitude to Drs. Karen Sprague, Richard Kramer, and Joan A. Steitz for helping me to master sequencing techniques. I would also like to thank Dr. William C. Summers who encouraged m to undertake this project. In addition, I would like to thank Larry Rubin for his technical assistance, and to acknowledge Dr. Jeremy Bruenn for his helpful suggestions.

References

Barrell, B. G. (1971), *Proced. Nucleic Acid Res. 2*, 751.
Bostock, C. J., Prescott, D. M., and Lauth, M. (1971), *Exp. Cell. Res. 66*, 60.
Dahlberg, J. E. (1968), *Nature (London) 220*, 548.
Dalgarno, L., and Shine, J. (1973), *Nature (London), New Biol. 245*, 261.
Dasgupta, R., Shih, D. S., Saris, G., and Kaesberg, P. (1975), *Nature (London) 256*, 624.
Eladari, M-E., and Galibert, F. (1975), *Eur. J. Biochem. 55*, 247.
Gorovsky, M. A., Meng-Chao, Y., Keevert, J. B., and Pleger, G. L. (1975), *Methods Cell Biol. 9*, 311.
Hunt, J. A., (1970), *Biochem. J. 120*, 353.
Legon, S. (1976), *J. Mol. Biol. 106*, 37.
McConkey, E. H. (1967), *Methods Enzymol. 12*, 620.
Sakuma, K., Kominami, R., and Muramatsu, M. (1976), *Eur. J. Biochem. 63*, 339.
Shine, J., and Dalgarno, L. (1974a), *Biochem. J. 141*, 609.
Shine, J., and Dalgarno, L. (1974b), *Proc. Natl. Acad. Sci. U.S.A. 71*, 1342.
Sprague, K. U., Kramer, R., Jackson, M. B. (1975), *Nucleic Acids Res. 2*, 2111.
Steitz, J. A., and Jakes, K. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72*, 4734.
Torriani, A. (1966), *Proced. Nucleic Acid Res. 1*, 224.