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Sequence of the 3'-terminal 21 nucleotides of yeast 17S ribosomal RNA

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ABSTRACT

The sequence of the 3'-terminal 21 nucleotides of 17S ribosomal RNA from the yeast Saccharomyces carlsbergensis has been determined to be (Y)G-m_2^6A-m_2^6A-C-U-C-G-C-G-G-A-A-G-G-A-U-C-A-U-U-A_{OH}. This sequence shows extensive homology with the 3'-terminal sequence of 16S rRNA from Escherichia coli including the presence of the two adjacent N^6-,N^6-dimethyladenosines observed in the small subunit rRNA of eukaryotes as well as of many prokaryotes.

INTRODUCTION

The 3'-terminal part of 16S rRNA from the small ribosomal subunit of bacteria has been shown to play an important role in the initiation of protein synthesis [1-3]. A cluster of pyrimidine nucleotides present near the 3'-end of 16S rRNA is complementary to a purine-rich nucleotide sequence found near the initiation codon of prokaryotic mRNA allowing the formation of a complex between the two RNAs. It has been proposed that a similar complex is formed between 17S/18S rRNA and mRNA during initiation of protein synthesis in eukaryotes [4-7]. Thus, the question arises to what extent the 3'-terminal sequence of the RNA from the small ribosomal subunit has been conserved during the course of evolution. So far several eukaryotic 17S/18S rRNAs have found to have the same 3'-terminal octanucleotide sequence G-A-U-C-A-U-U-A_{OH} [8-12], which can be aligned with the 3'-terminal sequence G-A-U-C-A-C-C-U-C-C-U-U-A_{OH} of E. coli 16S rRNA [13] assuming a deletion of the pentanucleotide C-C-U-C-C. It remains to be determined whether this conservation of primary structure extends further upstream and especially whether it includes conservation of the two consecutive m_2^6A residues found in the 3'-terminal part of E. coli 16S rRNA [13]. The sequence G-m_2^6A-m_2^6A-C seems to be universally present in eukaryotic 17S/18S rRNA [14,15].

Finally knowledge of the 3'-terminal structure of eukaryotic 17S/18S rRNA will also be important for understanding the mechanisms responsible for the final processing at the 3'-terminus of this RNA. In yeast this final

processing involves the removal of about 350 non-ribosomal nucleotides from the 3'-terminus of the immediate precursor of 17S rRNA[16] as well as methylation of the G-m_2^6A-m_2^6A-C sequence[17], which is possibly located near the 3'-end. Both processes take place in the cytoplasm.

In this paper we describe the determination of the sequence of the 3'-terminal 21 nucleotides of yeast 17S rRNA.

MATERIALS AND METHODS

Isolation of 17S rRNA

^{32}P-labelled 17S rRNA was isolated from a steady state culture of the yeast Saccharomyces carlsbergensis (strain N.C.Y.C. S-74) grown in a rich medium containing 0.1 mCi (^{32}P)orthophosphate per ml (The Radiochemical Centre, Amersham, P.B.S.-1). The RNA was purified via sucrose gradient centrifugation as described previously[16].

Digestion of RNA

About 1.5 x 10^8 dpm ^{32}P-labelled 17S rRNA (0.2 - 0.8 mg) was digested with 14 units of T_1 RNase (Calbiochem A.G. Lucerne, Switzerland) per mg of RNA for 20 min at 0°C in 100 µl 0.01 M Tris-HCl (pH 7.5) containing 0.001 M EDTA. The digestion was stopped by extraction with phenol/sodium dodecyl-sulphate. Residual phenol and SDS were removed from the sample by extraction with ether. The remaining ether was evaporated off under a stream of air.

Isolation of 3'-terminal RNA fragments

The sample containing the partial digest of 17S RNA was diluted with 9 volumes of solvent A[18] and subjected to DBAE-cellulose columnchromatography according to Rosenberg[18]. DBAE-cellulose (modified by acetylation of the non-boryl-substituted aminoethyl groups[19]) was purchased from Collaborative Research, Inc. (Waltham, Mass. 02154). The radioactivity in each fraction was determined by counting the Cerenkov radiation in a liquid scintillation counter. The appropriate fractions were pooled and desalted by extensive dialysis against distilled water.

After lyophilization the sample, which contains a mixture of 3'-terminal fragments of different lengths, was subjected to two-dimensional separation according to Brownlee and Sanger[20], using electrophoresis on cellulose acetate in 7 M urea at pH 3.5 in the first dimension and homochromatography on a thin layer DEAE-cellulose plate (polygram cel 300 DEAE/HR-2/15, 40 x 20 cm, Machery-Nagel + Co) at 65°C in the second dimension. The homomixture contained 3% (w/v) of yeast RNA, partially hydrolysed with 1 N

KOH for 9 min at 22°C. Spots were visualized by radioautography.

Analysis of oligonucleotides

The material present in the spots on the homochromatogram was eluted with 30% (w/v) triethylaminebicarbonate (pH 7.8). Each polynucleotide fragment was then digested to completion with T_1 RNase by incubation with 70 units of enzyme for 7 h at 37°C in 10 µl 0.01 M Tris-HCl (pH 7.5) containing 0.001 M EDTA. The digestion products were separated by electrophoresis on DEAE-cellulose paper in 7% formic acid. After radioautography the spots were excised and their radioactivity was determined in a gas flow counter.

Sequence analysis of the oligonucleotides was performed by standard techniques as described by Brownlee[21], using tertiairy digestion with pancreatic RNase (Sigma Chemical Co.,St. Louis, Mo.), U_2 RNase (Calbiochem) or 0.5 M KOH.

RESULTS

A set of successively longer polynucleotide fragments containing the 3'-terminus of an RNA species in principle can be obtained by partial nuclease digestion of the RNA followed by selective binding of the 3'-terminal fragments to a DBAE-cellulose column as described by Rosenberg[18]. The elution pattern of yeast 17S rRNA partially digested with T_1 RNase (Fig. 1) displays a large peak (designated A) of material which does not bind to the column as well as two smaller peaks (B and C) which are only eluted after changing the elution solvent. According to Rosenberg[18] peak B contains oligonucleotide material which has bound non-specifically to the dihydroxyborylgroups of the column whereas peak C comprises the fragments bearing free 2'- and 3'-OH groups at their 3'-terminus. The material present in peak C was pooled as indicated in Fig. 1 and subsequently fractionated two-dimensionally using high-voltage electrophoresis on cellulose acetate in the first dimension followed by homochromatography on a thin-layer plate of DEAE-cellulose in the second dimension. The resulting radioautogram (Fig. 2) shows the presence of four strong spots (1,3,5 and 6) as well as several weak ones. In other experiments, using slightly different digestion conditions, spot 2 and 4 were found to be more intense at the expense of spots 3 and 5. The background in the radioautogram is probably due to contamination with material from peak B (Fig. 1).

The material present in spots 1 through 6 was first checked for presence of the known 3'-terminal heptanucleotide sequence of 17S rRNA, A-U-C-A-U-U-A_{OH}[9,16]. The spots were eluted from the thin-layer plate and

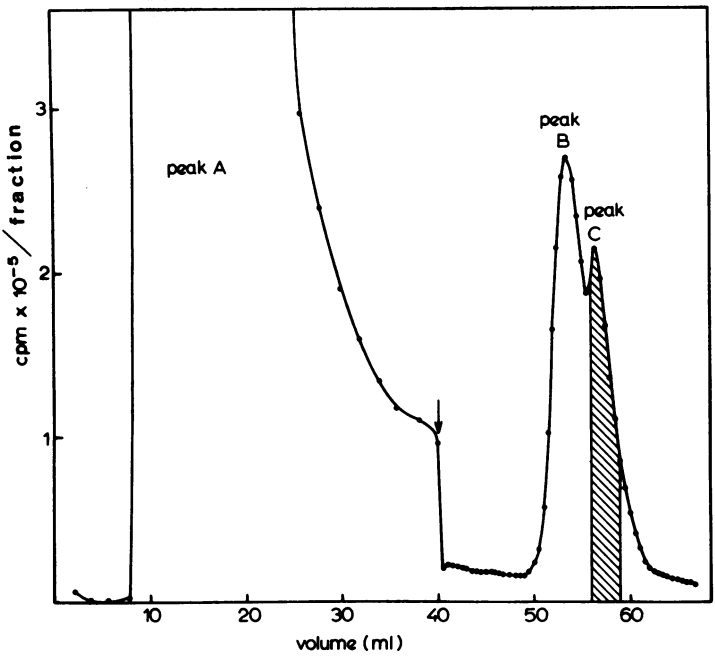
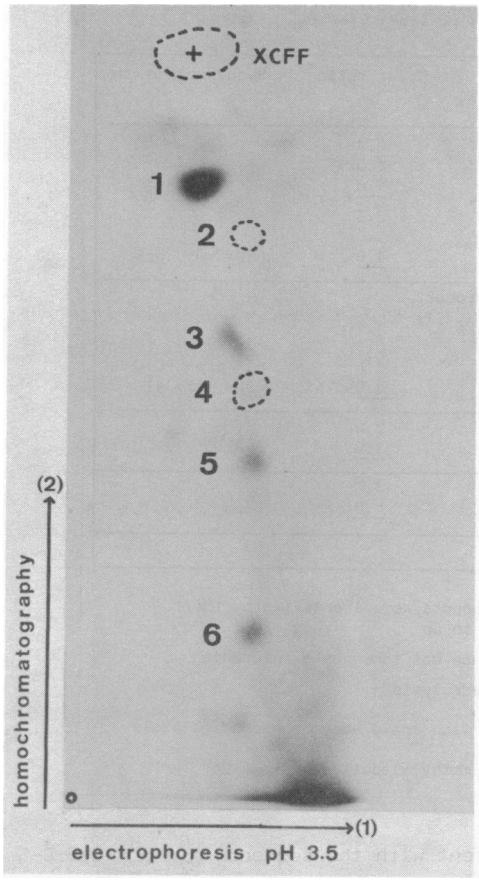
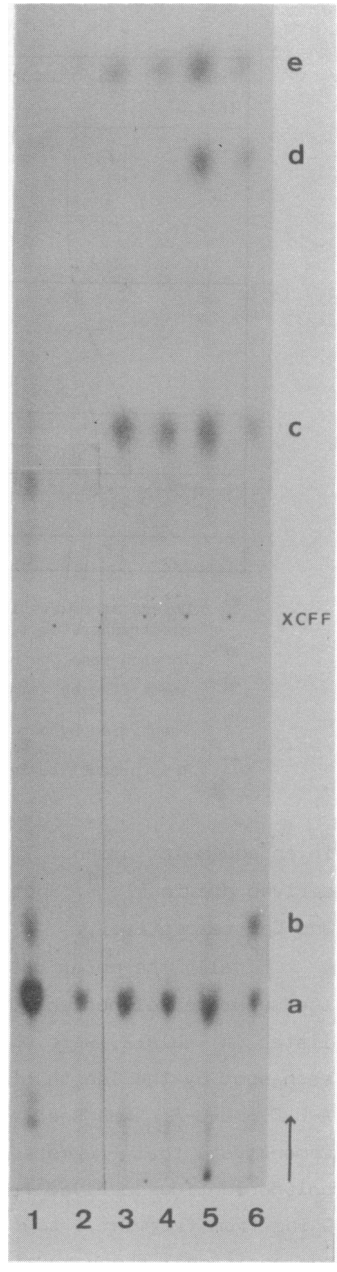


Figure 1. DBAE-cellulose column chromatography of a partial T_1 RNase digest of ^{32}P-labelled 17S rRNA. The arrow indicates the point at which solvent B^{18} was substituted for solvent A^{18}. The hatched area under peak C indicates the samples pooled for further analysis.

digested to completion with T_1 RNase. The digests were fractionated on DEAE-cellulose paper by electrophoresis in 7% formic acid. The radioautograph of this analysis (Fig. 3) shows that each of the spots gives rise to an oligonucleotide (spot a) having an electrophoretic mobility of 0.37 relative to the blue marker dye. This relative mobility is identical to that observed for the oligonucleotide A-U-C-A-U-U-A_{OH} in this electrophoresis system[16] indicating that spot 1 through 6 (Fig. 2) represent 3'-terminal fragments of 17S rRNA. Definite proof for this is obtained from the sequence analysis data shown in Table 1 which allow an unequivocal assignment of the sequence A-U-C-A-U-U-A_{OH} to spot a. The sequence of the remaining T_1 RNase digestion products of fragments 1 through 6 (Fig. 2), designated b-e in Fig. 3, were also determined by degradation with U_2 or pancreatic RNase or KOH and identification of the products. Table 1 summarizes the results obtained for the oligonucleotides derived from fragment 6. Identical results were observed for the oligonucleotides produced by the other five fragments. Spot b (Fig. 3) was found to contain m_2^6A-m_2^6A-C together with the nucleotides Cp, Up and Gp



(↑) Figure 2. Two-dimensional fractionation of the polynucleotide material present in peak C (Fig. 1). Marker dye: xylene cyanol FF. Spots 2 and 4 were too weak for photographic reproduction.



(→) Figure 3. Electrophoretic fractionation of the complete T_1 RNase digest of the polynucleotides from spot 1-6 (Fig. 2).

TABLE 1. IDENTIFICATION OF THE MAJOR T_1 RNase DIGESTION PRODUCTS OF THE 3'-TERMINAL FRAGMENT NO. 6 (FIG. 2) OF YEAST 17S rRNA

Product (Fig.3)	Digested with	Products obtained*)	Molar ratio**)	Sequence deduced
a	U_2 RNase	(U,C)$Ap^{\Pi)}_{\Gamma,\Pi)}$	0.9	
		U-U-A_{OH}	1.0	
		Ap	1.0	
	pancreatic RNase	A-Up	2.3	
		Cp	1.0	
		Up	1.0	A-U-C-A-U-U-A_{OH}
b	U_2 RNase	intact product		
	pancreatic RNase	m^6_2A-m^6_2A-$Cp^{\Pi,\dagger)}$	1.1	
		Gp	0.8	
		Cp	1.0	m^6_2A-m^6_2A-C-U-C-Gp
		Up	1.0	(see ref.14)
c	KOH	Ap	2.0	
		Gp	1.0	A-A-Gp
d	KOH	Cp	1.0	
		Gp	1.0	C-Gp
e	KOH	Gp	-	Gp

*) Unless otherwise indicated the products were identified by their electrophoretic mobility relative to Gp

**) In each case the underlined sequence was taken as a reference

Π) Identified by complete alkaline hydrolysis

Γ) Identified by complete hydrolysis with snake venom phosphodiesterase

†) This product comigrates with (^{14}C)methyl-labelled m^6_2A-m^6_2A-Cp

in an equimolar amount. This is consistent with the sequence m^6_2A-m^6_2A-C-U-C-G derived previously[14]. Spot c, d and e were found to represent A-A-Gp, C-Gp and Gp, respectively.

Finally the molar ratios of digestion products a through e, derived from the separate polynucleotide fragments 1-6, were determined. These data, listed in Table 2, were obtained by dividing the radioactivity present in each spot by the length of the respective digestion product. The sequence A-U-C-A-U-U-A_{OH} was used as a reference in each case. The results clearly demonstrate that fragments 1 through 6 form a series of successively longer polynucleotides derived from the 3'-terminus of 17S rRNA as shown in Table 3. Going from fragment 1 to fragment 6 each nucleotide sequence contains one additional cleavage site for T_1 RNase 5'-distal to the previous one. These results enable us to derive the sequence of the longest fragment (spot 6, Fig. 2) to be m^6_2A-m^6_2A-C-U-C-G-C-G-G-A-A-G-G-A-U-C-A-U-U-A_{OH}. Since in the complete pancreatic RNase digest of 17S rRNA the sequence

TABLE 2. MOLAR RATIOS OF THE T_1 RNase DIGESTION PRODUCTS OF 3'-TERMINAL FRAGMENTS NO. 1-6 (FIG. 2) OF YEAST 17S rRNA

Product (Fig. 3)	Sequence	molar ratios observed in the complete T_1 RNase digest of fragment*)					
		1	2	3	4	5	6
a	A-U-C-A-U-U-A_{OH}	1.0	1.0	1.0	1.0	1.0	1.0
b	m^6_2A-m^6_2A-C-U-C-Gp	< 0.1	-	-	-	-	1.1
c	A-A-Gp	< 0.1	-	1.0	1.1	0.9	0.9
d	C-Gp	-	-	-	0.1	1.0	0.9
e	Gp	-	1.2	1.2	2.0	2.2	1.9

*) A-U-C-A-U-U-A_{OH} was taken as a reference in each case

TABLE 3. NUCLEOTIDE SEQUENCE DERIVATION OF 3'-TERMINAL FRAGMENTS NO. 1-6 (FIG.2) FROM YEAST 17S rRNA

Fragment	Sequence
1	A-U-C-A-U-U-A_{OH}
2	G — A-U-C-A-U-U-A_{OH}
3	A-A-G — G — A-U-C-A-U-U-A_{OH}
4	G — A-A-G — G — A-U-C-A-U-U-A_{OH}
5	C-G — G — A-A-G — G — A-U-C-A-U-U-A_{OH}
6	m^6_2A-m^6_2A-C-U-C-G — C-G — G — A-A-G — G — A-U-C-A-U-U-A_{OH}
	b d e c e a

G-m^6_2A-m^6_2A-C was found to be present[14] the sequence of the 22 nucleotides located at the extreme 3'-end of 17S rRNA must be (Y)-G-m^6_2A-m^6_2A-C-U-C-G-C-G-G-A-A-G-G-A-U-C-A-U-U-A_{OH}.

DISCUSSION

The sequence of the last 21 nucleotides at the 3'-terminus of yeast 17S rRNA determined in this paper shows an extensive homology with the 3'-terminal sequence of *E. coli* 16S rRNA[13] including the presence of the two consecutive dimethylated A residues (Fig. 4). It is likely that this homology extends to 18S rRNA of higher eukaryotes. All eukaryotic 18S rRNAs studied so far have the 3'-terminal octanucleotide sequence G-A-U-C-A-U-U-A_{OH}[8-12]. Moreover, the sequence G-m^6_2A-m^6_2A-C also seems to be universally present in eukaryotic 17S/18S rRNA[14,15] and its location in eukaryotes may be expected to be the same as in yeast 17S rRNA.

The initiatior regions of several eukaryotic mRNAs, including Brome Mosaic Virus RNA[4], SV40-VP_1 RNA[5] and rabbit β-globin mRNA[6,7], have been

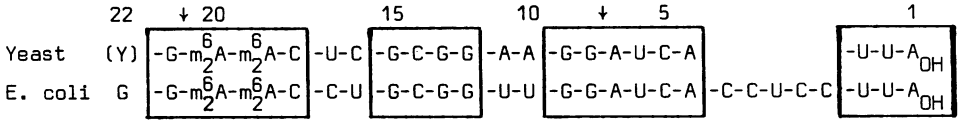


Figure 4. Sequence homology between the 3'-termini of yeast 17S and E. coli 16S rRNA. The arrows indicate bonds highly susceptible to T_1 RNase attack.

found to contain four to six bases complementary to part of the sequence G-A-U-C-A-U-U-A$_{OH}$ common to all eukaryotic 17S/18S rRNA species. This has prompted to the suggestion that, similar to the situation in E. coli, initiation of protein synthesis in eukaryotes involves base-pairing between the initiator region of mRNA and the 3'-terminus of the rRNA from the small ribosomal subunit[4,6,7]. The 21-nucleotide long 3'-terminal sequence of yeast 17S rRNA described in this paper does not show any significant additional complementarity to the initiator regions of the eukaryotic mRNAs mentioned. The putative base-pairing between mRNA and 17S/18S rRNA in eukaryotes, therefore, would be limited to part of the extreme 3'-terminal sequence of the rRNA similar to the base-pairing between prokaryotic mRNA and E. coli 16S rRNA[3].

Another feature of the 3'-terminal sequence of E. coli 16S rRNA is its potential to form an intramolecular hairpin containing the sequence G-m$_2^6$A-m$_2^6$A-C in its loop[2,13]. Some evidence for the existence of such a hairpin structure in vivo has been presented[2,22]. Our data on partial digestion with T_1 RNase suggest the existence of a similar secondary structure at the 3'-terminus of yeast 17S rRNA. Products 1 and 6 (Fig. 2, Table 3) were consistently found to be present in partial digests (prepared under somewhat different conditions) in amounts larger than those obtained for products 2-5 (unpublished data). The G residues at positions 8 and 21 (see arrows in Fig. 4) thus appear to be more readily accessible to T_1 RNase than those at positions 12, 13 and 15 (Fig. 4). This suggests that the latter residues are hydrogen-bonded to residues further upstream forming a hairpin structure containing both m$_2^6$A residues in its loop.

The biological function of the two consecutive m$_2^6$A residues still remains obscure. Recent experiments in our laboratory[17] demonstrated that methylation of the m$_2^6$A-m$_2^6$A site immediately preceeds the removal of about 350 nucleotides from the 3'-terminus of 18S precursor RNA, the final trimming step in the formation of mature 17S rRNA in yeast[16]. Since the methylation and trimming steps occur closely together both in time and space one might speculate that the latter is somehow dependent upon the former.

Alternatively the two dimethylated A residues may play a functional role in eukaryotic protein synthesis.

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