THE 3′-TERMINAL NUCLEOTIDE SEQUENCE OF THE 16 S RIBOSOMAL RNA FROM *ESCHERICHIA COLI*

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1. Introduction

We have recently been carrying out extensive studies of the primary structure of the 16 S ribosomal RNA from *Escherichia coli* (MRE 600) [1, 2]. In the course of this work we obtained a fragment from the 3′-end of this molecule, containing 40 nucleotides. This has been subjected to sequence analysis. We have found three methylated nucleotides within this fragment, although the frequency of modified nucleotides in the 16 S RNA is comparatively low [3, 4]. The possible significance of this cluster of modified nucleotides close to the 3′-terminus of the RNA is discussed.

2. Methods

Ribosomal 30 S subunits labelled with ^{32}P were prepared as previously described [5]. The subunits were partially digested with T_1 ribonuclease and the resulting fragments of RNA were extracted and fractionated by electrophoresis through a 6% polyacrylamide gel slab [2]. The fractionated products were detected by autoradiography, as shown in fig. 1. We examined a considerable number of the fragments by fingerprinting T_1 and pancreatic ribonuclease digests of them according to [6, 7]. The oligonucleotides were then identified by further enzymatic digestion, with reference to the maps of fingerprints of the 16 S RNA previously compiled [1, 8]. During this study, we encountered a fragment containing the oligonucleotide known to be present at the 3′-terminus of the 16 S RNA (see Results and discussion section).

3. Results and discussion

We originally reported that the 3′-terminal oligonucleotide, arising upon complete digestion of the 16 S RNA with T_1 ribonuclease, yielded AAAU, AU_2, AC_2, $(U+C)_4$ upon hydrolysis with pancreatic ribonuclease. It was also present in submolar amounts. However, it was clear upon subsequent re-examination of this spot that no AAAU is present. The partial sequence of this oligonucleotide was found to be AU(AC, C_4, U_3)A. The derivation of this sequence is described in the footnote to table 1. Presumably, in earlier fingerprints of the 16 S RNA, this spot had been contaminated by some other material containing AAAU. Additionally, this spot is now obtained in molar amounts. The original 16 S RNA used may have been slightly degraded, affording submolar quantities of the 3′-terminal oligonucleotide.

The products arising upon T_1 and pancreatic ribonuclease digestion of this fragment are listed in table 1. The corresponding fingerprint of the T_1 ribonuclease digestion products is shown in fig. 2. From the oligonucleotides arising upon complete hydrolysis with these enzymes, it is possible to deduce the complete sequence of the remainder of the fragment, without recourse to further partial enzymatic digestion. This is set out in fig. 3.

The most striking property of this sequence is the presence of three methylated nucleotides, containing five methyl groups. The functions of these methyl groups are not known. However, it has recently become clear that the 3′-terminal area of the 16 S RNA undergoes at least two types of specific interaction with proteins. First, it has been found [9] that the biosynthetic precursor of the 16 S RNA [10]

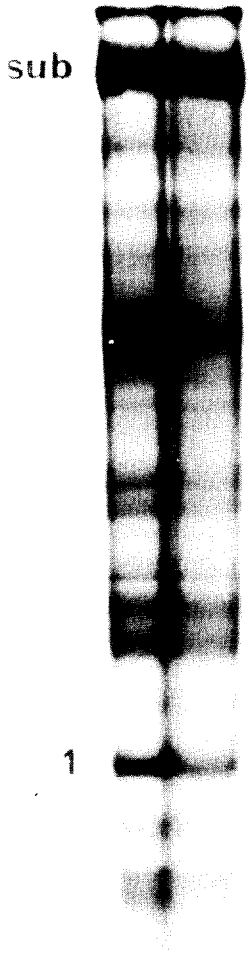


Fig. 1. Autoradiogram of products from partial digestion of 30 S ribosomal subunits fractionated by electrophoresis through polyacrylamide gel. The conditions of digestion have been described elsewhere [5]. Fragments were eluted electrophoretically [12]. The large fragment ('sub'), containing about 600 nucleotides, has also been partially characterised [5]. Band 1 contains the 3'-terminal fragment.

Table 1
Oligonucleotides arising upon T_1 and pancreatic ribonuclease digestion of fragment 1.

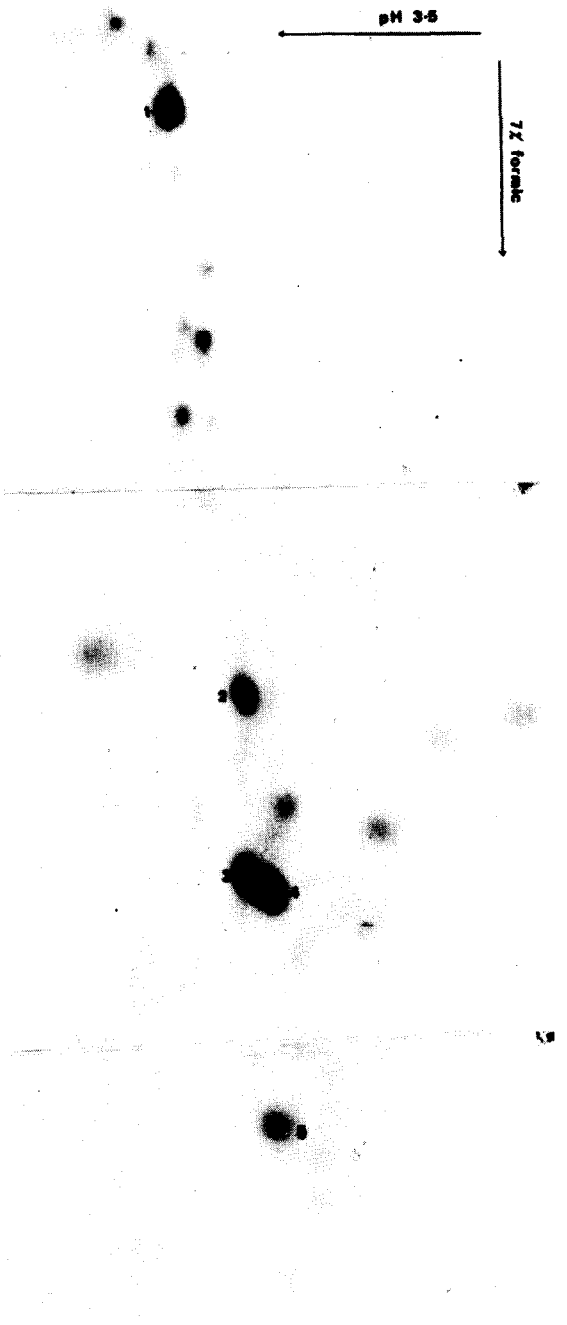
T_1 ribonuclease[a]	Pancreatic ribonuclease[b]
1) AU(AC,C_4,U_3)A	1) AGGGG$m_2^6Am_2^6$AC
2)[c] mUAACAAG	2) AAGGU
3)[d] $m_2^6Am_2^6$ACCUG	3) GGAU
4) CUAACG	4) mUAAC
5) UAG	5) GC
	6) AAC
	7) AC
	8) C
	9) U

[a] T_1 ribonuclease products were hydrolysed with pancreatic ribonuclease. The products were fractionated by electrophoresis on DEAE-paper at pH 1.9 [6]. From the pancreatic RNase products and the positions of the original spots, it was possible in all cases to identify the oligonucleotides, with reference to the map compiled earlier [1]. The sequence of the terminal oligonucleotide was partially deduced in the following way: pancreatic RNase digestion yielded AU,AC,U_3,C_4. Hydrolysis with alkali or venom phosphodiesterase (on the same spot from a fingerprint of the entire 16 S RNA) gave A_2,U_4,C_4 in both cases. From the pancreatic RNase product GGAU arising upon digestion of fragment 1, the AU must be placed at the 5'-terminus of this oligonucleotide, and the 3'-terminal nucleoside must be A.

[b] Pancreatic ribonuclease products were hydrolysed with T_1 ribonuclease, and the products were fractionated by electrophoresis on DEAE-paper at pH 1.9. On the basis of these analyses and the positions of the original spots, and also with reference to the T_1 ribonuclease digestion products from fragment 1, the complete sequences could be deduced.

[c] This methylated oligonucleotide was originally detected in fingerprints of ^{14}C-(methyl)-16 S RNA [3, 4]. The modified nucleotide was thought to be a derivative of uridylic acid, on the basis of its electrophoretic and chromatographic properties, but has not been completely identified. The modification of the nucleotide renders it resistant to the action of pancreatic ribonuclease. Pancreatic ribonuclease digestion of the entire 16 S RNA yields G(mU,A_2)C [8]. While pancreatic ribonuclease digestion of the T_1 ribonuclease oligonucleotide yields mainly (mU,A_2)C, some breakdown is observed (presumably because of the very large excess of enzyme under these digestion conditions) giving mU + AAC. This resolves the order of the nucleotides, and is confirmed by the absence of mU in venom phosphodiesterase digests of the same oligonucleotide from a fingerprint of the complete 16 S RNA. The oligonucleotide mUAAC is found among the pancreatic ribonuclease digestion products of fragment 1, indicating that this oligonucleotide must be at the 5'-terminus of this fragment.

[d] This methylated oligonucleotide was studied elsewhere [3, 4] and both the sequence and the identity of the modified nucleotides were determined. The unusual electrophoretic behaviour of m_2^6A-m_2^6A was also discussed [4]. The product m_2^6A-m_2^6AC is characteristic in that it has a substantially higher mobility than AAC during electrophoresis on DEAE-paper at pH 1.9.



$$G\overset{m}{U}AACAAGGUAGGGG\overset{6}{A}\overset{6}{A}CCUGCUAACGGAU(AC,U_3,C_4)A_{OH}$$

Fig. 3. The sequence of the 3′-terminal fragment.

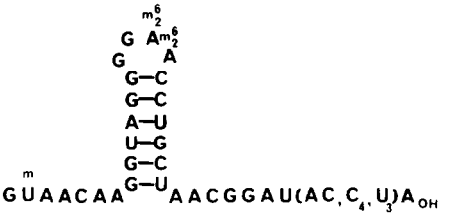


Fig. 4. Possible secondary structure existing within the 3′-terminal fragment.

contains additional nucleotides at the 3′-end, which are removed enzymatically during the maturation of the 16 S RNA. This precursor 16 S RNA also lacks the methylated nucleotides -m_2^6A-m_2^6A- [9]. It is likely that the methylated nucleotide mU is also absent, although this is not known with certainty. It might be suggested that the methylated nucleotides form part of the site recognized by the enzyme responsible for the trimming of the additional nucleotides from the 3′-end. The trimming would not occur before the methyl groups are introduced. A second possibility is that the methyl groups are involved in the binding site of one of the ribosomal proteins. Nomura [11] has recently reported that a defective 16 S RNA can be formed by the action of colicin E3 on susceptible cells. This lacks the 3′-terminal section and will not bind to the protein p15 during recon-

Fig. 2. A fingerprint of the products of T_1 ribonuclease digestion of fragment 1. After elution, band 1 was further fractionated by electrophoresis on cellulose acetate at pH 3.5 [12]. It was digested with T_1 ribonuclease and bacterial alkaline phosphatase [7], and fingerprinted according to Sanger et al. [6]. Despite the additional fractionation step on cellulose acetate, the fragment is not entirely pure. Low amounts of other oligonucleotides are visible. The numbers correspond to the sequences listed in table 1.

stitution of the 30 S ribosomal particle in vitro.

The sequence m_2^6A-m_2^6A- is extremely hydrophobic, and will not basepair with -U-U-. It must therefore be situated in a single-stranded portion of the 16 S RNA. From the sequence of the fragment shown in fig. 3, it is possible that it might contain a small amount of secondary structure, with the m_2^6A-m_2^6A sequence placed within a small loop. This is shown in fig. 4. It remains to be seen whether this sequence is able to participate in a more substantial secondary structure with some other part of the 16 S RNA.

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