THE 3′-TERMINAL NUCLEOTIDE SEQUENCE OF THE 23 S RIBOSOMAL RNA FROM *ESCHERICHIA COLI*

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1. Introduction

We have recently studied the nucleotide sequences of some of the products resulting from the digestion of the 23 S ribosomal RNA with T_1 ribonuclease. In the couse of this work we discovered an oligonucleotide having uridine as its 3′-terminal residue (spot 78, marked on fig. 1). It had previously been reported that the predominant nucleoside released by total alkaline hydrolysis of the 23 S RNA was uridine [1], implying that this nucleoside is present at the 3′-terminus of the molecule. We describe here the nucleotide sequence analysis of this product.

Table 1
The nucleotide sequence analysis of spot 78.

Material	Composition: [b] Alkaline hydrolysis	Composition: VPDE hydrolysis	[c] M Value	Suggested structure
Spot 78		$pC_2\ pA_2\ pU_4$		
RNase A Products: [a]				
a)				A-A-Cp
b)				Up_3
c)				Cp_2
Partial VPDE Products:				
a)	Cp Ap Up		1.9	C-U-U-A-A-C-C-U-U
b)	Cp Ap Up		1.7	C-U-U-A-A-C-C-U
c)	Cp Ap Up		0.1	C-U-U-A-A-C-C
d)	Cp Ap Up		0.2	C-U-U-A-A-C
e)	Cp Ap Up		0.4	C-U-U-A-A
f)	Cp Up		0.3	C-U-U-A
g)	Cp Up			C-U-U

[a] The pancreatic ribonuclease products were identified by their positions after electrophoresis on DEAE-paper at pH 1.9, in the company of marker oligonucleotides from other sequences. [b] Relative amounts of products were estimated by visual inspection of the film, as described in [3] and [4]. [c] See [2], [3] and [4] for discussion of M values.

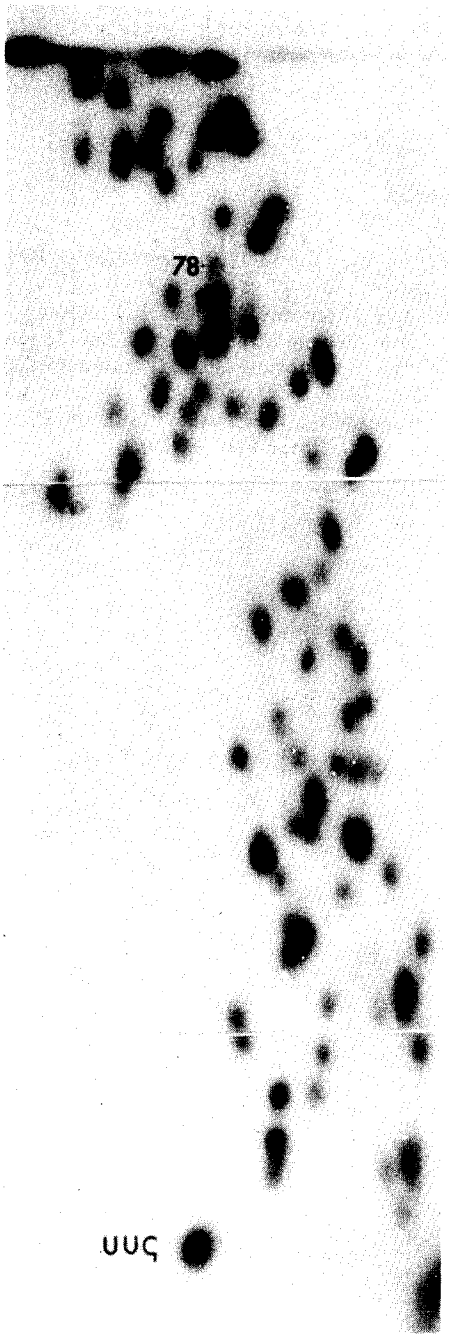

Fig. 1. A fingerprint of a T_1 ribonuclease digest of the 23 S RNA. A sample of 0.05 mg (about 0.4 mCi) of the 23 S RNA was digested with T_1 ribonuclease and bacterial alkaline phosphatase. The digest products were fractionated by electrophoresis on cellulose acetate at pH 3.5 (first dimension- right to left) and DEAE-paper in 7% formic acid (second dimension-top to bottom). An autoradiogram of the resulting fingerprint is shown above. In this case, the oligonucleotide U-U-G was allowed to migrate about 75 cm away from the origin in the second dimension, to permit an adequate fractionation of the larger oligonucleotides.

2. Methods

^{32}P-labelled 23 S RNA was prepared from *E. coli* (MRE 600) as previously described [2]. The material was digested with T_1 ribonuclease and bacterial alkaline phosphatase [2,3] and the products were fractionated by high-voltage electrophoresis in 2 dimensions [4]. The nucleotide sequence analysis of spot 78 was carried out by examining the products of hydrolysis with pancreatic ribonuclease and venom phosphodiesterase, and of partial digestion with venom phosphodiesterase. The methods of digestion and of fractionation of the products have been previously described [3,4]. In this case, partial digestion of the oligonucleotide with a solution of 0.02 mg/ml venom phosphodiesterase, for 10 min at room temperature, was suitable. The products were fractionated by electrophoresis on DEAE-paper at pH 1.9.

3. Results and discussion

The results obtained are summarised in table 1. On the basis of these findings we believe that the 3′-terminal decanucleotide sequence of the 23 S RNA is:

G-C-U-U-A-A-C-C-U-U

It cannot be excluded that spot 78 might arise from elsewhere within the molecule by degradation of the RNA before or during isolation. However, more than 30 other large oligonucleotides arising from T_1 ribonuclease digestion of the molecule have been examined, and they all contain 3′-terminal guanosine. Also, our unpublished quantitative studies indicate that spot 78 is present in roughly equimolar amounts (0.7–0.8 moles) with many other large oligonucleotides arising in T_1 ribonuclease digests of the molecule. In addition, 0.4–0.5 moles of spot 78 are liberated per mole of both of the methylated sequences A-A-A-T-U-C-C-U-U-G and A-C-A-U-A-U-m^1G-ψ-T-G, which are believed to be present twice within the molecule [2,5].

The 5′-terminal sequence of the 23 S RNA is reported to be pG-G-U [6]. This could participate in base-pairing with the -A-C-C- portion of the supposed 3′-terminal fragment; this may be coincidental, since the above trinucleotide sequence would be expected to occur at least 30 times within a random polynucleotide of the size and composition of 23 S RNA. Therefore, there is a 10% the chance of it occurring within the terminal decanucleotide.

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