SEQUENCE ANALYSIS OF THE 3′-T_1 OLIGONUCLEOTIDE OF 16S RIBSOMAL RNA FROM *ESCHERICHIA COLI*

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1. Introduction

In our recent papers [1,2], the sequence of the 3′-T_1 oligonucleotide of *Escherichia coli* 16S ribosomal RNA was not completely established and only a partial sequence was given. Two conflicting sequences of this region have since been proposed by Santer and Santer [3] and Shine and Dalgarno [4]. The latter authors reported that the 3′-end sequence is complementary to ribosome binding sequences found in intercistronic regions of a number of coliphage mRNA species, as well as with terminator codons, and suggested that it could be involved in the initiation and termination processes. Therefore, it appeared to us necessary to determine unambigously this 3′-terminal sequence. The use of U2 ribonuclease and venom phosphodiesterase allowed us to deduce the sequence A–U–C–A–C–C–U–C–C–U–U–A_{OH}, which confirms the partial sequence proposed by Shine and Dalgarno [4].

2. Materials and methods

We used ^{32}P-labelled 16S RNA from *E. coli* MRE 600 prepared as previously described [5]. The methods of T_1 digestion (in presence of bacterial alkaline phosphatase) and the fractionation of the resulting oligonucleotides have been fully described by Sanger et al. [6] and Brownlee and Sanger [7]. In order to obtain a better resolution of the 3′-T_1 oligonucleotide (10b according to the numbering system of Fellner et al. [1]), we carried out a long electrophoresis in the second dimension.

Digestion with U2 ribonuclease (Sankyo) was carried out in 10 μl of a solution containing 1 unit/ml U2 ribonuclease, 0,05M NH_4 acetate pH 4.5, 5 mg/ml cold tRNA carrier, for 15 hr, at 37°C. The fractionation of the resulting products was performed on DEAE-paper, in 7% formic acid. Other methods of analysis used are fully described elsewhere [6,7].

3. Results and discussion

The products obtained by pancreatic ribonuclease (see table 1) had been characterised previously and it was already known that A–U is located in 5′-position [8]; the partial sequence could therefore be written: A–U(A–C,U_3,C_4)A_{OH}. U2 ribonuclease digestion was then carried out and spots 14 (UCAUCAUCG) and 34 (AUCAUG) were chosen to give position markers of U–C–Ap and Ap. Three products, U2–1, U2–2 and U2–3 were obtained (see table 1) and subjected to total digestion by pancreatic ribonuclease and venom phosphodiesterase (VDPE). The presence of Ap (U2–1) confirms the location of A–U in the 5′-position. The presence of 5′-AMP among the venom phosphodiesterase products and the absence of 3′-AMP among the pancreatic products of the oligonucleotide U2–3 (see table 1) show clearly that the terminal 3′-hydroxyadenosine is contained in the oligonucleotide U2–3. From these results, we can deduce the following partial sequence: A–U–C–A–C(C_3,U_3)A_{OH}.

Partial venom phosphodiesterase was then carried out on oligonucleotide U2–3 and the digest was fractionated on DEAE-paper at pH 3.5. The resulting oligonucleotides were subjected to total venom phosphodiesterase hydrolysis. The M values, introduced

Table 1
Nucleotide sequence analysis of the 3′-T_1 oligonucleotide of 16S ribosomal RNA from *E. coli*

1 - Pancreatic ribonuclease products		$Up_3,Cp_4,A-Cp,A-Up$			
		pancreatic ribonuclease products	VDPE products	partial VDPE products	M value pH 3.5
2 - U2 ribonuclease products	1 Ap				
	2 U–C–Ap	Up,Cp,Ap			
	3 C–(C_3,U_3)A_{OH}	Up_3,Cp_4	pU_3,pC_3,pA	C–C–U	
				C–C–U–C	0.7
				C–C–U–C–C	0.8
				C–C–U–C–C–U	1.9
				C–C–U–C–C–U–U	1.9
				C–C–U–C–C–U–U–A_{OH}	2.2

by Sanger et al. [6], have been calculated and are indicated in table 1. The inspection of these results allowed us to determine unambigously the following sequence: A–U–C–A–C–C–U–C–C–U–U–A_{OH} (see fig. 1). This sequence is in disagreement with the sequence A–U–C–C–U–C–A–C–U–U–C–A_{OH} reported by Santer and Santer [3], but confirms the partial sequence by A–C–C–U–C–C–U–U–A_{OH} reported by Shine and Dalgarno [4].

Further experiments will now be necessary in order to ascertain the role of this 3′-terminal sequence in the initiation or termination processes in protein synthesis, since up to now no direct evidence has been provided.

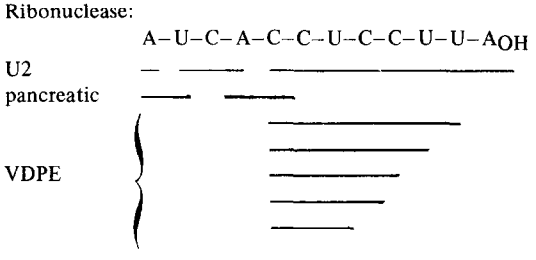

Fig. 1. Deduced sequence of the 13′-T_1 oligonucleotide of *E. coli* 16S RNA. The lines indicate the oligonucleotide obtained by digestion with the different ribonucleases used.

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