SEGMENTS OF THE 16 S RNA LOCATED ON THE SURFACE OF THE 30 S SUBUNITS OF *ESCHERICHIA COLI* RIBOSOMES

N. L. TETERINA, A. M. KOPYLOV and A. A. BOGDANOV

A. N. Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow 117234, USSR

Received 28 May 1980

1. Introduction

Ribosomal RNA directly participates in the ribosome functioning being involved in interactions with mRNA [1,2], tRNA [3], initiation and elongation factors [4,5] as well as in the association of ribosomal subunits [6–8]. However, despite the functional importance of rRNA almost nothing is known about the spatial organization of rRNA in ribosomes and the data on the surface topography are still very scanty. The identification of single guanine residues [7–9] and single phosphodiester bonds [10,11] accessible to chemical modification and RNase T_1 hydrolysis, respectively, has been attempted. The data obtained in [7–11] do not give, however, any idea about the length of the exposed regions of rRNA. At the same time it is of interest to identify rather extended RNA sequences on the subunit surfaces since they can be involved in organizing the binding sites for the components of the translational apparatus.

We have developed an approach which allows one to release from the surface of the 30 S subunit of *E. coli* ribosomes from 10–15% of the 16 S RNA in the form of mono- and oligonucleotides under the conditions in which the subunits retained their compact structure and protein composition [12,13]. This paper reports the results of analysis of the primary structure of exterior segments of the 16 S RNA in the small ribosomal subunit. In particular, the data obtained in this study provide information about the macromolecular organization of a 150-nucleotide 3′-terminal segment of the 16 S RNA, an important functional site of the 30 S ribosomal subunit.

2. Materials and methods

2.1. *Materials*

Pancreatic RNase and phosphodiesterase from snake venom were products from Worthington; RNase T_1 was obtained from Sankyo Inc. (Tokyo); alkaline phosphatase was purchased from Sigma; T4 polynucleotide kinase was a generous gift of Dr B. Yuodka; [γ-^{32}P]ATP with spec. act. 400 Ci/mmol was a generous gift of Dr K. Skrjabin. Polygram CEL 300 PEI thin-layer plates (Serva) were prepared for chromatography as in [14]. Cellulose acetate Cellogel (Chemetron Milano) was used for electrophoresis at pH 3.5. Sephadex G-10 and G-50 were from Pharmacia Fine Chemicals (Uppsala).

2.2. *Preparation of the fractions of exposed oligonucleotides*

30 S subunits from *E. coli*, strain MRE 600 were prepared as in [12]. Subunits were dissolved in a buffer consisting of 10 mM Tris–HCl, 100 mM KCl, 10 mM $MgCl_2$, 6 mM 2-mercaptoethanol, and incubated for 30 min at 30°C for activation. 30 S subunits were digested in the same buffer either with pancreatic RNase (8 μg enzyme/1 mg subunits) or with RNase T_1 (0.5 μg enzyme/1 mg subunits) for 3.5–4 h at 25°C. Part of the reaction mixture was used for determination of the amount of the ethanol-soluble fraction [12] and the remaining solution was applied on Sephadex G-50 column for separation of the oligonucleotide fraction from digested subunits. The oligonucleotide fraction was concentrated and desalted by gel-filtration on Sephadex G-10 column.

2.3. *Analysis of oligonucleotides*

0.1 A_{260} units of oligonucleotides in 5 μl 10 mM Tris–HCl buffer (pH 8.5) were treated with alkaline

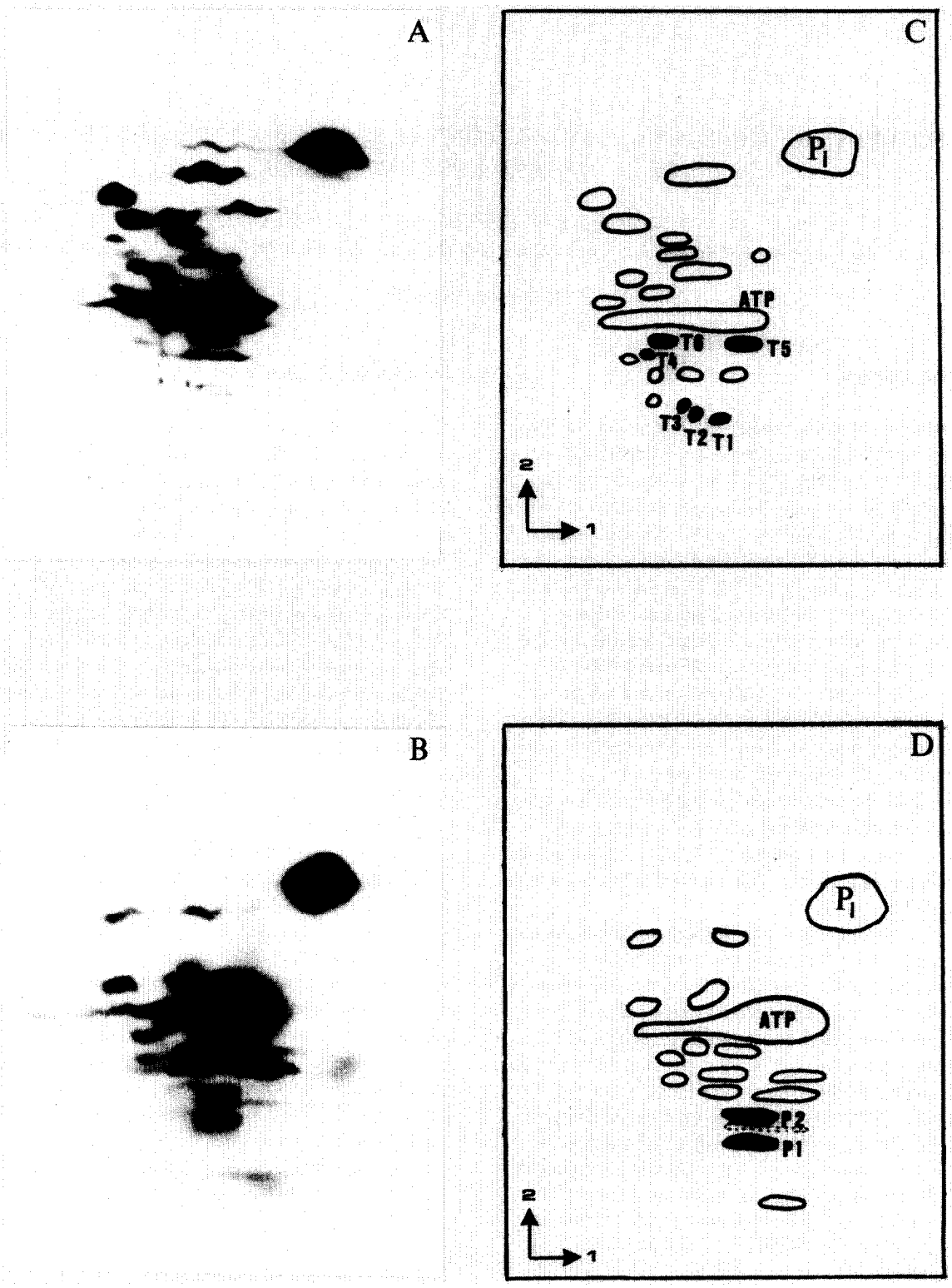

Fig.1A,B,C,D

phosphatase for 30 min at 30°C to remove the 3′-end phosphate group. The enzyme was inactivated with EDTA (added to 6.5 mM) and then with heating of the solution for 3 min at 90°C. The buffer (1 μl) consisting of 1 M Tris–HCl (pH 8.0), 200 mM $MgCl_2$, 200 mM 2-mercaptoethanol, 40 mM spermidine, 0.2 μl 10% solution of diethylpyrocarbonate, 4 μl T4-polynucleotide kinase and 200 μCi [γ-^{32}P]ATP were added to the reaction mixture. The mixture was incubated for 30 min at 37°C and then submitted to two-dimensional separations; i.e., first dimension, electrophoresis on cellulose acetate at pH 3.5; second dimension, homochromatography [14]. Spots containing ^{32}P radioactivity were scraped off the plates, oligonucleotides were eluted and submitted to partial digestion with snake venom phosphodiesterase in a buffer consisting of 10 mM Tris–HCl (pH 8.0), 10 mM $MgCl_2$, at 37°C. Digestion products were separated in a two-dimensional system [14]. 5′-End mononucleotide residue was determined by paper electrophoresis on Whatman 3 MM, at pH 3.5, after complete digestion of oligonucleotide with snake venom phosphodiesterase.

3. Results and discussion

The oligonucleotides, released from the 30 S subunits of *E. coli* ribosomes by RNase treatment, were labeled at their 5′-end with [γ-^{32}P]ATP and T4-polynucleotide kinase and then separated by two-dimensional electrophoresis/homochromatography technique. More than 70% of the released nucleotide material were discovered in the form of mono- and short oligonucleotides which were not unique in the 16 S RNA chain. However, several rather large oligonucleotides were detected among the products of hydrolysis (fig.1). Their structures were determined by the method in [15], which included partial digestion of the ^{32}P-labeled oligonucleotide with snake venom phosphodiesterase and two-dimensional electrophoresis/homochromatography separation of the hydrolysate. The structure of a nonanucleotide released from 30 S subunits as a result of pancreatic RNase treatment has been determined in a previous study using a classical technique [13]. We have failed to determine primary structures of several relatively long oligonucleotides due to their being not homogeneous enough. The results are summarized in table 1.

Since the sequences of all oligonucleotides presented in table 1 are unique in the 16 S RNA molecule we were able to locate them unambiguously in the RNA chain. Oligonucleotides T1, T2, T3, T4 and P1 appear to be in the 3′-end proximal region of the

Table 1
Oligonucleotides released from the 30 S subunits by RNase T_1 and A treatment

Oligonucleotides[a]	Structure	Location in the 16 S RNA[b]
T1	CUUACCACUUUG	1461–1472 section A[c]
T2	CUUAACCUUCG	1442–1452 section A
T3	UCACACCAUG	1405–1414 section A
T4	AUUCAUG	1475–1481 section A
T5	UUUUCAG	1006–1012 section D
T6	CUCUUUG	87– 93 section H
PI	AAAAGAAGU	1427–1435 section A
P2	GAAGAGU	6– 12 section L

[a] T1–T2, oligonucleotides released with RNase T_1; P1 and P2, oligonucleotides released with pancreatic RNase

[b] The primary structure of the 16 S RNA in [16] has been used to locate the oligonucleotides

[c] Letters refer to sections generally used in 16 S RNA sequencing works [17]

Fig.1. Separation of ^{32}P-labeled oligonucleotides: A,B, radioautographs of two-dimensional separations of oligonucleotides released from the 30 S subunits with RNase T_1 and pancreatic RNase treatment, respectively; C,D, schematic diagrams indicating the positions of oligonucleotides T1–T6, and P1,P2, respectively.

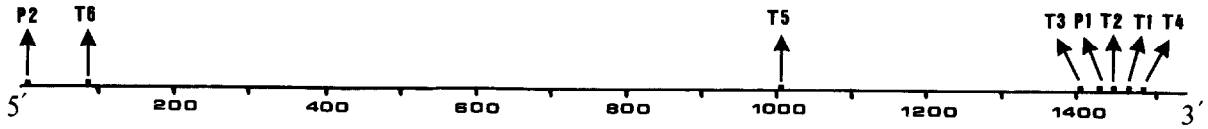

Fig.2. The positions of the exterior segments of the 16 S RNA which can be released from the 30 S subunits with RNase treatment in form of oligonucleotides.

16 S RNA (fig.2). They represent the major part of sequence 1405–1481 in a 150-nucleotide 3′-terminal segment of the 16 S RNA, an important site of the small ribosomal subunit which is involved in many functional interactions [2,7,8,11]. This result is in good correlation with the general concept of the exterior location of the 3′-end area of the 16 S RNA [10,11]. In addition, it allows one to conclude that a large 3′-proximal segment of the 16 S RNA is not involved in any strong interactions with ribosomal proteins or other parts of the RNA molecule. A large portion of this segment can be removed from the 30 S subunits without any marked alteration in their sedimentation coefficients or protein composition. It is also interesting that the secondary structure of this segment is apparently highly conserved in 16 S ribosomal RNA of prokaryotic organisms [11].

The oligonucleotide T5 locates in the middle of the 16 S RNA chain, whereas oligonucleotides T6 and P2 are parts of its 5′-end proximal region (fig.2). The guanine residues accessible to modification by kethoxal have been found in close proximity of T6 and P2 [9,11]. At the same time, among the oligonucleotides located in the 3′-end proximal region of the 16 S RNA only T3 has the neighbouring G-residue which can be readily modified in the 30 S subunit. This demonstrates that determination of rRNA regions exposed on the surface of ribosomal subunits depends to a great extent on the nature of the agent used.

In conclusion, we emphasize that 8 exterior RNA segments identified here represent either single-stranded portions of the 16 S RNA or its regions with very weak secondary structure. This fact has to be taken into account when considering models of 16 S RNA secondary structure in 30 S subunits.

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