Nucleotide Sequence and in Vitro Processing of a Precursor Molecule to Escherichia coli 4.5 S RNA*

(Received for publication, June 21, 1976)

ALFRED L. M. BOTHWELL,* RICHARD L. GARBER, AND SIDNEY ALTMAN§

From the Department of Biology, Yale University, New Haven, Connecticut 06520

A precursor molecule to the stable 4.5 S RNA species of Escherichia coli has been found to accumulate at 42° in a strain thermosensitive for the function of ribonuclease P. The precursor molecule is 130 nucleotides long. Twenty-two extra nucleotides, starting with pppGp, precede the mature sequence at its 5' terminus. At least 1 extra uridine residue can be found at the 3' terminus. The precursor to 4.5 S RNA is cleaved in vitro by RNase P to generate a 5' end identical to that of the mature 4.5 S RNA.

Several low molecular weight RNAs which have no known function have been identified in Escherichia coli. One of these is designated 4.5 S RNA because of its electrophoretic mobility in polyacrylamide gels. The primary structure of this RNA has been determined by Griffin (1). The 4.5 S RNA is very stable in vivo and the quantity in a bacterial cell is comparable to that of a single tRNA species. It contains no modified nucleotides and does not appear to be associated with ribosomes.

The accumulation of 4.5 S RNA in vivo, as for tRNA and 5 S rRNA, is under stringent control (2). A precursor to 4.5 S RNA has been tentatively identified by other investigators at restrictive temperature in strains of E. coli thermosensitive for RNase P function (3, 4). Since both the transcription and the processing of transcripts from genes coding for 4.5 S RNA are apparently under the same control as the genes and gene transcripts for tRNA, it is possible that 4.5 S RNA plays some role in protein synthesis. In order to study in detail one aspect of this control, endonucleolytic processing by RNase P, we have determined the nucleotide sequence of a 4.5 S RNA precursor molecule which accumulates in E. coli strain A49 at restrictive temperatures (4). Even though the structure of the mature portion of this molecule is different from that of the tRNA portion of tRNA precursor RNAs, it is in need a substrate for RNase P. In fact, the availability of the products generated both by RNase P and human KB cell RNase NU cleavage of the 4.5 S RNA precursor molecule has greatly facilitated the nucleotide sequence studies. Furthermore, determination of the cleavage sites in 4.5 S RNA precursor of these enzymes has provided interesting new information concerning the factors governing specific ribonuclease-substrate interactions and the function of the "extra" transcribed sequences in RNA processing.

EXPERIMENTAL PROCEDURE

Materials

Bacterial Strains—Escherichia coli A49 (RNase P is) was a gift from P. Primakoff and P. Schedl (4). E. coli MREG600 (RNase I-) was used to prepare RNA P.

Tissue Culture—Human KB cells were grown in monolayer cultures in Eagle's Minimal Essential Medium (Flow Laboratories, Rockville, Md.) supplemented with 10% (v/v) calf serum (Grand Island Biological Co., Grand Island, N.Y.), nonessential amino acids, and 292 μg/ml of L-glutamine.

Enzymes—Pancreatic DNase (DNPP), RNase A, bacterial alkaline phosphatase, venom phosphodiesterase, and spleen phosphodiesterase were purchased from Worthington Biochemical Corp., Freehold, N.J. RNases T1 and T2 were obtained from Calbiochem La Jolla, Calif. RNase U1 was a gift from J. Steitz of Yale University. RNase P was prepared according to the methods of Robertson et al. (5). RNase NU was prepared as described by Bothwell and Altman (8) except that the ribosomes were washed in the 0.2 M NH4Cl buffer containing 0.002 M magnesium acetate instead of 0.01 M magnesium acetate.

TPGA Medium—This medium contained 0.5 g of NaCl, 8.0 g of KCl, 1.1 g of NH4Cl, 0.4 g of MgCl2-6H2O, 0.022 g of Na2SO4, 0.8 g of sodium pyruvate, and 12.1 g of Tris base in 1 liter of water. The following supplements are autoclaved separately and added to 180 ml of the above medium: 0.2 ml of 1 mg/ml of vitamin B12, 2 ml of 20% (w/v) glucose, 0.1 ml of 0.1 M KH2PO4, and 20 ml of 3% (w/v) dephosphorylated vitamin-free casein acids (Difco). L-Tryptophan is added to the medium at a final concentration of 20 μg/ml immediately preceding the inoculation of a culture.

Chemicals—All routine chemicals were of reagent grade. Ultrapure ammonium sulfate and sucrose were purchased from Schwarz/Mann, Orangeburg, N.Y. Carrier free [*P]PO4 was purchased from New England Nuclear Corp., Cambridge, Mass. CMCT was a gift from S. Endow of Yale University.

Radioactive Polyribonucleotides—[32P]labeled M13 RNA (7), 4 S RNA, and 5 S tRNA were prepared as described by Bothwell and Altman (6, 8). The [32P]-labeled precursor to 4.5 S RNA was prepared as follows. A culture of strain A49 was grown at 30° to a density of about 108 cells/ml.
4 × 10^6 cells/ml in TPGA medium. The cells were shifted to 42°C for 45 min and then labeled with 5 to 10 mCi of carrier-free 32P0,3- for 20 min. The RNA was extracted by the addition of an equal volume of 88% phenol at room temperature, followed by vigorous shaking for 10 min. The RNA was precipitated with ethanol and then separated in and extracted from 10% polyacrylamide slab gels as described previously (9).

**Methods**

Nucleotide Sequence Analysis—Sequence analysis was performed according to the methods developed by Sanger and collaborators (10, 11). CMCT blocking was done according to Barrell (12). When alkaline phosphatase and CMCT blocking were used together, the substrate was first incubated in 5 µl of 0.1 mg/ml of alkaline phosphatase in 0.05 M Tris/HC1 (pH 8.9) and 0.01 M MgCl2 and then heated for 3 min at 90°C to inactivate the alkaline phosphatase. The sample was dried, resuspended in the CMCT blocking buffer, and processed according to Barrell (12).

**RESULTS**

Identification of Precursor to 4.5 S RNA—A labeled RNA species having the same mobility in 10% polyacrylamide gels as the φ80 psu3- phage-encoded tRNA,$^7$ precursor (9) can be found in 32P-labeled RNA preparations made at restrictive temperatures in uninfected Escherichia coli A49 cells (Fig. 1). Fingerprint analysis shows that this species (Fig. 3, panel A) contains all of the RNase T1 oligonucleotides characteristic of mature 4.5 S RNA (1), except that it lacks the mature 5'-pGp terminus. Furthermore, this RNA can be cleaved in vitro by purified RNAse P to yield two products: a molecule which is the same size as 4.5 S RNA and a smaller fragment 22 nucleotides in length (Fig. 2, lane 6). Finally, no modified nucleotides were found in the RNA after digestion with RNase T2 and two-dimensional thin layer chromatography. Griffin found this to be the case for mature 4.5 S RNA (1). These observations identified the molecule as a precursor to 4.5 S RNA.

Preparations of the 4.5 S RNA precursor also contain two tRNA precursors amounting to about 10% of the total radioactive RNA. This was judged by the relative amounts of 4.5 S and tRNA-size products resulting from RNase T cleavage of the precursor band. Therefore, the intact precursor is at least 90% radiochemically pure as eluted from a gel. RNase NU, an endoribonuclease isolated from human KB cells, can also cleave the 4.5 S RNA precursor to yield fragments 111 and 19 nucleotides long (Fig. 2, lane 7). Because their size and electrophoretic mobilities differ from the cleavage products of the contaminating tRNA precursors, the RNase P and RNase NU-derived fragments of the 4.5 S precursor are radiochemically pure. This greatly facilitated the determination of the primary sequence of the "extra" part of the precursor molecule.

Nucleotide Sequence Analysis of Precursor to 4.5 S RNA—Fingerprints of the RNase T1 and RNase A digests of the 4.5 S RNA precursor and of the products of RNase P and RNase NU cleavage of the precursor are shown in Fig. 3. Panel A shows a RNase T1 fingerprint of the intact precursor, while panel B gives the fingerprint of the 4.5 S RNA generated by RNase P digestion of the precursor for comparison. The fingerprints look identical because several of the precursor-specific oligonucleotides coincidentally co-migrate with oligonucleotides found in the mature molecule. Characterization of the precursor-specific sequences required separation of the 5' extra piece from the mature segment by digestion of the precursor with RNase P or RNase NU.

Some information concerning the extra sequence in the 4.5 S RNA precursor can be obtained by comparing the molar yields of the RNase T1 oligonucleotides from the intact precursor with the yields from the 4.5 S fragment resulting from RNase P cleavage of the precursor. Increased molar yields of products 1, 2, 15 and 16 (see Table I) were observed in the intact precursor compared with those found in the part of the molecule destined to become mature 4.5 S RNA. The product CpApAppGp, found in 4.5 S RNA from strain MREG00 (1), has never been observed in the 4.5 S RNA precursor or the mature 4.5 S RNA from strain A49. However, an extra mole of CpApAppGp was found. This product was probably the result of a guanosine occurring at position 98 instead of an adenosine (see legend to Table I).

Griffin has observed some variation in the molar yield of certain RNase T1 and RNase A products in separate preparations of mature 4.5 S RNA from a single E. coli strain. This was especially true for RNase T1 and RNase A products containing the sequence AppGp. Similar variation has been detected in the mature segment of RNA derived from RNase P cleavage of the 4.5 S precursor. The values in Table I are, therefore, the average molar yields from several preparations.
The RNase T₁ products 2 to 4 were readily identified by their electrophoretic mobilities and by secondary analysis using RNase A. Determination of the sequences of oligonucleotides 5, 6, and 7 required more extensive analysis.

**Determination of Sequence of Oligonucleotide 5 (see Fig. 3A)**—After RNase A treatment, T₁, oligonucleotide 5 yielded Up, Cp, ApApCp, and Gp which were present in the molar ratio 2:2:1:1. RNase A digestion can be restricted by chemical blocking of uridylic acid residues with CMCT. CMCT blocking and subsequent RNase A digestion of oligonucleotide 5 yielded UpUUpCp, ApApCp, Cp, and Gp. In some experiments where blocking was incomplete, as indicated in reactions with known oligonucleotides treated in parallel experiments, a small amount of UpCp was also evident.) RNase U₂ digestion of oligonucleotide 5 produced a large fragment ending in Ap, as well as CpGp and Ap. This information indicated that the four 3'-proximal nucleotides were ApApCpGp and that only pyrimidines could be present in the 5' side of this sequence in oligonucleotide 5. Thus, the sequence was assigned as (UpUUpCp, Cp) ApApCpGp. Partial spleen phosphodiesterase treatment (Table III) showed that the 5' terminus was Up. The sequence of oligonucleotide 5 is therefore UpUUpCp-CpApApCpGp.

**Determination of Sequence of Oligonucleotides 6 and 7 (see Fig. 3D)**—Oligonucleotide 6 results from RNase T₁ digestion of the 5' RNase P-generated fragment. Since it contains no Gp it must be located at the 3' end of this fragment. RNase A digestion yielded Up, Cp, and ApApUoH in yields of 1:3:1. The sequence ApApUoH was deduced from the products of alkaline hydrolysis (Ap only) and venom phosphodiesterase digestion (pA and pU). Thus, oligonucleotide 6 must have four pyrimidines followed by ApApUoH. After CMCT blocking and RNase A digestion of oligonucleotide 6, the products were Up, Cp, and ApApUoH, making the sequence (UpApU, Cp) ApApUoH.

Ordering the pyrimidines in oligonucleotide 6 was made easier because RNase NU (which produces fragments terminating with 3' phosphates) cleaved the intact precursor between the fourth pyrimidine and the ApApUoH sequence in this oligonucleotide. Oligonucleotide 7 in Fig. 3, panel E, contained the pyrimidine tetranucleotide. Alkaline hydrolysis of oligonucleotide 7 produced Up and Cp in a 1:3 ratio. CMCT blocking and RNase A digestion of this oligonucleotide produced UpCp and Cp in a 1:2 ratio. From analysis of partial RNase T₁, cleavage products (discussed in detail below) it was deduced that Cp must be the 5' nucleotide. The fourth nucleotide must also be Cp as the only uridine residue was present in the dinucleotide UpCp. Therefore, there were two possible sequences for this tetranucleotide: CpUpCp or CpCpUpCp. This ambiguity was resolved by treating the tetranucleotide with alkaline phosphatase to remove the 3'-phosphate. This enzyme was then heat-inactivated and the oligonucleotide treated with CMCT and subsequently RNase A. If the tetranucleotide had the sequence CpCpUpCp, then the removal of the 3'-terminal phosphate should give the product CpCpUpCp. Treatment with CMCT and then RNase A should not result in the detection of UpCp. However, the products Cp and UpCp were obtained with or without alkaline phosphatase treatment. Thus, the sequence of RNase NU oligonucleotide 7 is CpUpCpCp and that of RNase P oligonucleotide 7 is CpUpCpCp and that of RNase P oligonucleotide 7 is CpUpCpCp.

**Precursor RNA to E. coli 4.5 S RNA**

---

**Fig. 2.** Comparison of the cleavage products of the RNase P and RNase NU reactions on three RNA substrates. The reactions were performed as described previously (6). The RNase P used in these experiments was the DEAE-Sephadex fraction described by Robertson et al. (5) and the RNase NU was the ammonium sulfate fraction (6). The cleavage products obtained by RNase NU digestion of the 4.5 S RNA precursor or the M₄ RNA were the same using either the ammonium sulfate fraction or the more pure DEAE-Sephadex fraction. The substrate used in the experiments shown in lanes 1 to 3 was the 6 S tRNA precursor indicated in Fig. 1 and is shown here only as a mobility marker which can also be cleaved by RNase P; to lane 4, the mobility markers 4 S RNA, 5 S RNA, and 6 S tRNA precursor were added; in lanes 5 to 7, the substrate used was 4.5 S RNA precursor and in lanes 8 to 10 it was M₄ RNA which is shown for comparison (14). The three-nucleotide size difference between the 4.5 S RNA precursor cleavage products produced by RNase P and RNase NU appears as a mobility difference in lanes 6 and 7. The two contaminating tRNAs produced by RNase P cleavage of the 4.5 S RNA precursor preparation are visible in lane 6.

---

**Note:** The text above is extracted from a scientific paper discussing the cleavage products of RNA by RNase P and RNase NU, focusing on the mobility differences and sequence determination of specific oligonucleotides.
FIG. 3. Fingerprints of the 4.5 S RNA precursor and the products of cleavage obtained from digestion with Escherichia coli RNase P and human KB cell RNase NU. Panels A to E are fingerprints of the products of RNase T digestion and panel F is a fingerprint of the products of RNase A digestion. Panel A, intact 4.5 S RNA precursor with the 5' terminal T, product labeled pppGp. Panel B, the 4.5 S RNA produced by RNase P cleavage of the 4.5 S RNA precursor; note the presence of pGp, the 5' terminus of mature 4.5 S RNA (1). Analysis of an RNase A digest of the RNase P-generated mature 4.5 S RNA sequence showed that the 5'-terminal oligonucleotide was pGpGpGpGpCp, indicating clearly that RNase P cleavage produced the correct 5' terminus. Panel C, the large product of cleavage of 4.5 S RNA precursor by RNase NU (see Fig. 2, lane 7); note the new 5' terminal product, ApApUpGp, the two 3' terminal products which streak in the second dimension and the absence of pGp (see text). Panel D, the RNase P-generated 5' fragment of the 4.5 S RNA precursor. The products are numbered to correspond to the numbers in Table II. Panel E, the RNase NU-generated 5' fragment of the 4.5 S RNA precursor. The products are numbered to correspond to those listed in Table II. Panel F, the RNase P-generated 5' fragment of 4.5 S RNA precursor. The identity of the products has been indicated. The RNase NU-generated 5' fragment has an identical RNase A fingerprint except it lacks the product labeled ApApUu. In each fingerprint, separation was by electrophoresis on cellulose acetate in pyridine acetate, 7 M urea, 0.001 M EDTA, pH 3.5, from right to left, and on DEAE-paper in 7% formic acid (v/v), from top to bottom.
Further analysis of this product revealed the presence of ApGp, RNase A, and RNase U, digests of these products gave the products accomplished only by the use of RNase P and RNase NU to produce the fragments containing the 5' sequence alone (see Table II).

The numbering of the T, products corresponded to the products labeled in Fig. 3, panels D and E. The molar yields were expressed relative to CpGp or GpUp taken as 1.0. CMCT blocking, followed by RNase A digestion, was used to locate the positions of uridine in oligonucleotides 5, 6, and 7. Products of digestion were separated by one-dimensional electrophoresis on Whatman No. 3MM paper, at pH 3.5. CMCT-modified products migrated toward the cathode and are given a positive sign relative to the origin; unmmodified products migrated toward the anode and are given a negative sign. Product identity (after removal of blocking group) is given with migration distances: UpUpGp = +15.4 cm; Gp = +13.3 cm; UpGp = +10.4 cm; ApGpGp = +8.6 cm; Gp = -2.8 cm; ApApGp = -15.3 cm; unblocked Gp = -26.6 cm. The final composition of these products was determined by alkali digestion and analysis of the products of this treatment.

---

**Table I**

| T, Product | Sequence | Cleaved 4.5 S RNA | Precursor 4.5 S RNA |
|------------|----------|------------------|---------------------|
| 1          | UpUpGpCpGpApApUp | 0.7              | 1.4                |
| 2          | CpGp       | 1.0              | 1.3                |
| 3          | CpUpGpCpGp  | 1.0              | 0.6                |
| 4          | GpGp       | 0.6              | 0.4                |
| 5          | UpGp       | 2.3              | 0.6                |
| 6          | ApGpGp     | 1.9              | 1.3                |
| 7          | CpUpGp     | 1.0              | 1.0                |
| 8          | GpGp       | 1.2              | 1.0                |
| 9          | UpGpGp     | 1.3              | 0.8                |
| 10         | UpUpGp     | 1.0              | 1.0                |
| 11         | CpAUpGpCpGp| 1.5              | 2.0                |
| 12         | ApGpGp     | 2.1              | 1.8                |
| 13         | ApGp       | 3.4              | 3.1                |
| 14         | CUpGp      | 1.0              | 1.3                |
| 15         | GpGp       | 1.0              | 0.7                |
| 16         | Gp         | 12.4             | 35.0               |
| 17         | 3' end     | 0.6             | 0.6*              |
| 18         | ppppGp     | 0.0              | 0.4*              |

*a* The sequence of these products was determined by Griffin (1). RNase A and RNase U, digests of these products gave the products expected from the indicated sequence. No further verification of their sequence was done.

The radioactivity found in this spot in the precursor contained 4.4 mol of octanucleotides. The separation of the large oligonucleotides found in the extra 5' sequence in the 4.5 S RNA precursor was expected from the indicated sequence. No further verification of their identity (after removal of blocking group) is given with migration distances: UpUpGp = +15.4 cm; Gp = +13.3 cm; UpGp = +10.4 cm; ApGpGp = +8.6 cm; Gp = -2.8 cm; ApApGp = -15.3 cm; unblocked Gp = -26.6 cm.

---

**Table II**

| RNase A produced oligonucleotides | 5' RNase P Fragment | 5' RNase NU Fragment |
|----------------------------------|---------------------|----------------------|
|                                  |                     |                      |
| 1 ppppGp                          | 0.9* 1              | 1 ppppGp             | 0.8* 1 |
| 2 CpGp                           | 1.0 1               | 2 CpGp               | 1.0 1  |
| 3 GpGp                           | 1.0 1               | 3 Gp                 | 1.0 1  |
| 4 UpUpGp                         | 1.0 1               | 4 UpUpGp             | 0.9 1  |
| 5 UpUpCpCpApApGpGp               | 0.9* 1              | 5 UpUpCpCpApApGpGp   | 0.8* 1 |
| 6 CpUpCpCpCpApApGpApUp           | 1.0 1               | 6 CpUpCpCpCpApGpApUp | 0.8 1  |

*a* The values in this column are experimental.

---

Cp, blocked Gp, and a small amount of unblocked Gp. The only product obtained with phosphatase treatment was Cp.) Complete Sequence of 5' Fragment—The complete sequence of the extra 5' fragment of the 4.5 S precursor molecule was defined through analysis of the products obtained from a partial RNase T, digestion of the RNase P-produced 5' fragment. The products of the partial digestion (designated t, to t,) were separated by homochromatography (Fig. 5) and further characterized in the standard fashion. The composition of product t, shows that the RNase A product GpUp (Table IV) must connect the RNase T, products 2 and 4 (Table II). The composition of products t, and t, indicates that the product GpUp (Table II) must connect complete RNase T, products 3, 4, and 5. Similarly, t, and t, show that GpGp (Table II) must connect RNase T, products 5 and 6 (Table II). These data were then used to construct the complete sequence of the 5 fragment shown in Fig. 6.

As shown by the determination of the sequence of oligonucleotide 7, the RNase NU cleavage site is located between nucleotides 19 and 20 of the precursor (Fig. 7). Consequently, the large RNase NU cleavage product, which contains the mature 4.5 S RNA sequence, should have a 5'-terminal sequence of ApApUApUp. This oligonucleotide was indeed found in the appropriate RNase T, fingerprint (Fig. 3C) and its identity was confirmed by secondary analysis with RNase A. As expected, there was no Gp in this fingerprint.
TABLE III

Analysis of products from spleen phosphodiesterase partial digestion of oligonucleotide 5 (UpUpCpCpApApCpGp)

Spleen phosphodiesterase partial digestion was carried out as described by Brownlee (16) and the products separated in 7% \( (v/v) \) formic acid on DEAE-paper. Two oligonucleotides were isolated and then treated with CMCT and RNase A (see "Experimental Procedure") and the mobilities of these further products correlated with known oligo- or mononucleotides. Other oligonucleotides could not be recovered in sufficiently large amounts for further analysis.

| Partial spleen product | Products obtained after CMCT treatment followed by RNase A digestion |
|------------------------|---------------------------------------------------------------------|
| UpCpCpApApCpGp         | UpCp, Cp, ApApCp, Gp                                               |
| CpApApCpGp             | Cp, ApApCp, Gp                                                     |

Griffin (1) found sequence heterogeneity in preparations of the mature 4.5 S RNA and suggested that more than one gene for this RNA must exist. It has been possible to separate two bands of radioactivity when the 4.5 S RNA precursor is rerun on a 20% polyacrylamide gel (3) (data not shown). Although the fingerprints of these two species appeared identical it is not yet possible to say whether the separation was the result of heterogeneity in the primary sequence or the existence of two conformations of 4.5 S RNA precursor.

Analysis of 3' Terminus of Precursor to 4.5 S RNA—The two RNase T, products from the precursor containing the 3'

![Figure 4](image-url)  
Fig. 4. Analysis of the 5' terminus of the 4.5 S RNA Precursor (18). Lane 1 shows the products of alkaline hydrolysis of M, RNA, which has pppGp at its 5' terminus. The position where pppAp would migrate has been indicated; lane 2 contained the product labeled 7 in Fig. 3, panel D and has the same mobility as pppGp. Lane 3 contained pppGp which was eluted from the fingerprint shown in Fig. 3, panel D. This spot represented about 20% of the 5' terminus of the precursor. 0 indicates the origin of electrophoresis which was on DEAE-paper in 0.05 M sodium citrate, pH 4.0, from bottom to top.

![Figure 5](image-url)  
Fig. 5. Products of partial ribonuclease T, digestion of the RNase P-produced 5' fragment of the 4.5 S RNA precursor. The digestion was performed at an enzyme:substrate ratio of 1:1000 for 15 min at 0°. Partial products are designated t1 to t4 and the complete digestion products numbered 2 to 6 are the same products listed in Table II. Separation was by electrophoresis on cellulose acetate in pyridine acetate, 7 M urea, 0.001 M EDTA, pH 3.5, from right to left, and by homochromatography on a DEAE-cellulose thin layer chromatography plate with the "homo C" solution (16) from top to bottom.
Precursor RNA to E. coli 4.5 S RNA

TABLE IV
Analysis of oligonucleotides produced by RNase T1, partial digestion of RNase P-generated 5' fragment of 4.5 S RNA precursor molecule

The partial products listed are those identified in Fig. 5. Each partial product was isolated and further analyzed by complete digestion with the enzymes indicated using standard techniques.

| RNase T1 partial digestion product | Products isolated after further digestion with RNase T1 or RNase A |
|----------------------------------|---------------------------------------------------------------|
| t1                               | pppGp, ApCp, UpGpUpGp, UpCp, ApUpCp, ApUpGp, ApUpUpGp        |
| t2                               | pppGp, ApGp, UpGpUpGp, UpCp, ApUpCp, ApUpGp, ApUpUpGp        |
| t3                               | ApUpUpCp, ApUpCp, ApUpGp, UpCp, ApUpCp, ApUpGp, ApUpUpGp     |
| t4                               | ApUpUpCp, ApUpGp, UpCp, ApUpCp, ApUpGp, ApUpUpGp, ApUpUpGp   |

Fig. 6. Nucleotide sequence of the RNase P-generated 5' fragment of the 4.5 S RNA precursor molecule. The sequence is constructed using the results of the analysis of oligonucleotides produced by RNase T1. Partial digestion of the fragment (lines below the sequence; see Fig. 5 and Table IV) and of RNase T1 (top line above the sequence) and RNase A (second line above the sequence) complete digestion (see Fig. 3 and Table II).

The 5' terminus (Fig. 3, panel C) were subjected to RNase A digestion and found to contain ApCp and Cp residues as well as variable amounts of Up. In a separate experiment, when a RNase T1 digest of the large fragment of RNase P digestion of 4.5 S RNA precursor molecule was examined using homochromatography, two distinct products were seen which contained 3'-terminal sequences. Neither of these products yielded Up after RNase A digestion, but the product moving faster in the pH 3.5 electrophoresis cellulose acetate step yielded pU after snake venom phosphodiesterase digestion. Thus, it is apparent that at least one additional uridine residue may be present at the 3' terminus of the precursor molecule and the occasional presence of more than one 3'-terminal uridine residue in vivo cannot be excluded.

Use of 4.5 S RNA Precursor Fragments for Studies of Enzyme Specificity—The mature 4.5 S RNA cannot be cleaved by either RNase P or RNase NU. When the RNase P-produced 5'-proximal fragment (which still contained the RNase NU cleavage site) was prepared, it was not further cleaved by RNase NU. Similarly, the RNase NU cleavage product of the 4.5 S RNA precursor which contains the mature 4.5 S RNA sequence plus three extra nucleotides at the 5' terminus (and thus the RNase P site) was not further cleaved by RNase P. After this substrate was exposed to RNase P, it was repurified by gel electrophoresis and analyzed by digestion with RNase T1. The RNA yielded a RNase T1 fingerprint identical to the one shown in Fig. 3, panel C. The intact 5'-terminal of this RNase T1-generated product, ApApUpGp, was still observed and no pGp was apparent. Thus, no nucleotides were removed from either end of the molecule.

DISCUSSION

The precursor to the 4.5 S RNA has several interesting features. The 5' terminus begins with pppGp, showing that this molecule is a gene transcript unaltered at its 5' terminus. The sequence proximal to the 3' terminus of the molecule indicates it may be a remnant of transcription termination (7, 17, 18) since it can terminate with at least one and possibly more uridine residues. If the 3' terminus in the 4.5 S RNA precursor...
Precursor RNA to E. coli 4.5 S RNA

is the result of in vivo transcription termination, then the maturation of the precursor to 4.5 S RNA might be accomplished by RNase P and only one other enzyme, perhaps a 3’-to-5’-exonuclease.

The nucleotides in the 5’-proximal “extra” sequence of the 4.5 S RNA precursor molecule can be drawn in the hydrogen-bonded structure shown in Fig. 7. The hairpin structure is stable as judged by the simple rules derived by Gralla and Crothers (19). In fact, when limiting amounts of RNase T1 are used to digest the molecule, cleavage of the 5’ hairpin occurs only in the loop of the hairpin, as expected from the hypothetical secondary structure scheme.

The overall structure of the 4.5 S RNA precursor molecule is remarkably similar to that of bacteriophage φ80-induced M4 RNA (7) which also has a 5’-proximal small hairpin and a much larger 3’-proximal hairpin connected by a short single-stranded region. These two RNAs share other features. Both 4.5 S precursor RNA and M4 RNA are cleaved in vitro by RNase P at the junction of a single-stranded region and the long 3’-proximal hairpin (14). In the case of 4.5 S RNA precursor, the large cleavage product is the mature 4.5 S RNA molecule. The function of the cleavage products of M4 RNA is not known nor is it certain that RNase P cleaves M4 RNA in vivo. It is of interest that both 4.5 S precursor RNA and M4 RNA have some sequence similarity which spans eight nucleotides around the RNase P cleavage site (14). The possible hydrogen-bonded structures of M4 RNA and 4.5 S RNA precursor and their primary structure around the cleavage sites distinguishes them from tRNA precursors as a separate type of RNase P substrates. In considering the two types of RNase P substrates, a hypothesis has been put forth concerning the cleavage site specificity of RNase P in which several nucleotides on the 5’ side of the cleavage site are necessary for directing enzyme attack on M4 RNA and 4.5 S precursor RNA but not on tRNA precursors (14). Indeed, since RNase P cannot cleave the large RNase NU-produced fragment containing the mature 4.5 S RNA sequence, several extra nucleotides at the 5’ terminus of the 4.5 S RNA precursor must be essential for the normal maturation of 4.5 S RNA. We do not know if, in vivo, RNase P cleavage requires trimming of a 3’-terminal extra sequence which has yet to be found.

Several possible functions of 4.5 S RNA have been considered (1). One was that 4.5 S RNA might be a by-product of rRNA maturation. This can now be ruled out since the 5’ terminus of the large 30 S rRNA precursor molecule is pppAp (20) which differs from the 5’ terminus of the 4.5 S RNA precursor molecule.

Since 4.5 S RNA biosynthesis is under stringent control and processing of its precursor molecule requires RNase P function (as is the case with tRNAs), it is tempting to link the function of the mature molecule to some aspect of protein synthesis. In considering what role this molecule might play, and in what ways its structural similarity to M4 RNA may be relevant, we propose that the long double-stranded hairpin generated by RNase P cleavage in each case plays a regulatory role in protein synthesis, perhaps in interactions with specific initiation factors. Interactions between double-stranded RNA molecules and factors controlling protein synthesis have been proposed to account for some features of the regulation of protein synthesis in eukaryotes (21).

REFERENCES
1. Griffin, B. E. (1975) J. Biol. Chem. 250, 5426-5437
2. Ikemura, T., and Dahlberg, J. E. (1973) J. Biol. Chem. 248, 5035-5041
3. Sakano, H., Yamada, S., Ikemura, T., Shimura, Y., and Ozeki H. (1974) Nucleic Acid Res. 1, 355-371
4. Schedl, P., Primackoff, P., and Roberts, J. (1974) Brookhaven Symp. Biol. 26, 53-76
5. Robertson, H. D., Altman, S., and Smith, J. D. (1972) J. Biol. Chem. 247, 5243-5251
6. Bothwell, A. L. M., and Altman, S. (1975) J. Biol. Chem. 250, 1451-1459
7. Piecznik, G., Barrell, B. G., and Gefter, M. L. (1972) Arch. Biochem. Biophys. 152, 152-165
8. Bothwell, A. L. M., and Altman, S. (1975) J. Biol. Chem. 250, 1460-1463
9. Altman, S. (1971) Nature New Biol. 229, 19-21
10. Sanger, F., Brownlee, G. G., and Barrell, B. G. (1965) J. Mol. Biol. 13, 373-388
11. Brownlee, G. G., Sanger, F., and Barrell, B. G. (1968) J. Mol. Biol. 34, 379-412
12. Barrell, B. G. (1971) in Procedures in Nucleic Acid Research (Cantoni, G. L., and Davis, D. R., eda) vol. 2, pp. 751-779, Harper and Row, New York
13. Schedl, P. (1975) Ph.D. thesis, Stanford University
14. Bothwell, A. L. M., Stark, B. C., and Altman, S. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 1912-1916
15. Konrad, M. W., Toivonen, J., and Nierlich, D. P. (1972) Nature New Biol. 238, 231-233
16. Brownlee, G. G., and Sanger, F. (1969) Eur. J. Biochem. 11, 356-406
17. Bertrand, K., Korn, L., Lee, F., Platt, T., Squires, C. L., Squires, C., and Yanofsky, C. (1975) Science 189, 22-23
18. Lebowitz, P., Weissman, S. M., and Raddine C. M. (1971) J. Biol. Chem. 246, 5120-5139
19. Gralla, J., and Crothers, D. M. (1973) J. Mol. Biol. 73, 391-411
20. Ginsburg, D., and Steitz, J. A. (1975) J. Biol. Chem. 250, 5647-5654
21. Robertson, H. D., and Mathews, M. B. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 225-229
Nucleotide sequence and in vitro processing of a precursor molecule to Escherichia coli 4.5 S RNA.
A L Bothwell, R L Garber and S Altman

J. Biol. Chem. 1976, 251:7709-7716.

Access the most updated version of this article at http://www.jbc.org/content/251/23/7709

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/251/23/7709.full.html#ref-list-1