Abnormal maturation of precursor 16S RNA in a ribosomal assembly defective mutant of E. coli

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ABSTRACT

The precursor and mature 16S ribosomal RNAs from a novel thermosensitive ribosomal assembly defective mutant of E. coli, in which genetic evidence suggests that the ribosomal protein S4 is altered, have been isolated and characterised by finger-printing methods. The precursor 16S RNA, which is accumulated at 42°, appears to be identical with that present in wild-type strains, and with that previously described by other workers. However, the mature 16S RNA, which is contained in apparently normal functional 30S ribosomal particles synthesised at the growth-permissive temperature of 30°, is incompletely trimmed and has either one or two additional nucleotides at its 5'-terminus. This might be due either to an accumulation of two late intermediates in the maturation process, or to mis-trimming of the RNA. Both possibilities suggest that the change in the protein S4 is not only responsible for the thermosensitive character of ribosomal assembly in this mutant, but also causes an alteration in the trimming site, affecting its recognition by the enzyme involved in the maturation.

INTRODUCTION

The mature rRNAs found in functional bacterial ribosomes arise by the specific enzymatic cleavage of larger precursor molecules. Various authors have used fingerprinting methods to study the additional nucleotides present in a precursor of the 16S RNA (p16S RNA) isolated either from pulse-labelled exponentially growing cells[1,2,3], chloramphenicol-treated cells[4], or a cold-sensitive mutant defective in ribosomal assembly[4]. These nucleotides were detected in fingerprints of the precursor RNAs as characteristic extra products.

Furthermore, the usual 5'- and 3'-terminal oligonucleotides of the mature 16S RNA (m16S RNA) were absent from fingerprints of the precursors, being included instead in two of the larger additional oligonucleotides.

We have used sequencing methods to investigate 16S RNA maturation in a novel thermosensitive strain of E. coli K12, termed

219, carrying the mutation sud_2. Genetic evidence has been obtained suggesting that in this mutant the ribosomal protein S4 in the 30S ribosomal subunit is altered. At 42° this strain is defective in 30S subunit assembly and accumulates a precursor particle of about 26S. This particle contains a p16S RNA which has been shown to be somewhat larger than the m16S RNA by polyacrylamide gel electrophoresis[5]. We report here that the sequence of this precursor is in fact virtually identical to the precursor species described by other authors[4]. However, the subsequent enzymatic trimming of this p16S RNA is incomplete, and we find that the apparently normal functional 30S particles synthesises at 34° contain an m16S RNA which is either one or two nucleotides longer than usual.

METHODS

(a) Preparation and purification of m16S RNA and p16S RNA from E. coli strain 219:

20 ml of TB medium (Bacto Tryptone 1.3%, NaCl 0.7%) was inoculated with 0.2 ml of an overnight culture. Growth at 34° was followed by the A_{420mu} in a Coleman spectrophotometer. When the A_{420mu} reached 0.15, the culture was divided into two parts, and treated in the following way:

(i) 15 ml of the culture was incubated at 42°. After 15 min at this temperature, 8 mCi of $H_3{}^{32}PO_4$ (10 mCi/ml, obtained carrier-free from the Commissariat d'Energie Atomique, Saclay) were added. The incubation was continued for a further 75 min at 42°.

(ii) 5 ml of the culture was kept at 30°, and 2 mCi of $H_3{}^{32}PO_4$ were added. The incubation was continued for 60 min, followed by a chase for a further 60 min achieved by adding 10 mg/ml of unlabelled KH_2PO_4.

At the appropriate times, the cells from both incubations were harvested by centrifugation, and washed with 10 ml of TMA_I buffer (10 mM tris HCl, pH 7.8; 30 mM NH_4Cl; 10 mM $MgCl_2$; 6 mM -mercaptoethanol). The bacteria were then resuspended in 5 ml of this buffer and disrupted in a French pressure cell at 12,000 psi, in the presence of 20 ug/ml of DNase. Unbroken cells and debris were removed by centrifugation for 20 min at 30,000 g, and the RNAs were extracted from the supernatant by deproteinisation[7]. The RNAs were precipitated with ethanol at -10°, and recovered by centrifugation. The m16S and p16S RNAs were then purified by electrophoresis on an

agarose-acrylamide composite gel according to Dahlberg et al.[8] The bands containing the purified RNA species were detected by auto-radiography, and were then excised and eluted according to Jordan[9]. The RNAs were finally freed of any contaminating acrylamide by precipitation with perchloric acid, as described by Brownlee and Cartwright[3].

The RNAs were digested with T_1 ribonuclease in the presence of bacterial alkaline phosphatase and the products were fingerprinted as described by Brownlee and Sanger[10]. Further digestion of oligonucleotides with pancreatic ribonuclease, and fractionation of the resulting products by electrophoresis on DEAE-paper at pH 1.9 was carried out according to Sanger et al.[11] The 5'-terminal oligonucleotide of the p16S RNA was isolated using the diagonal method described by Dahlberg[12].

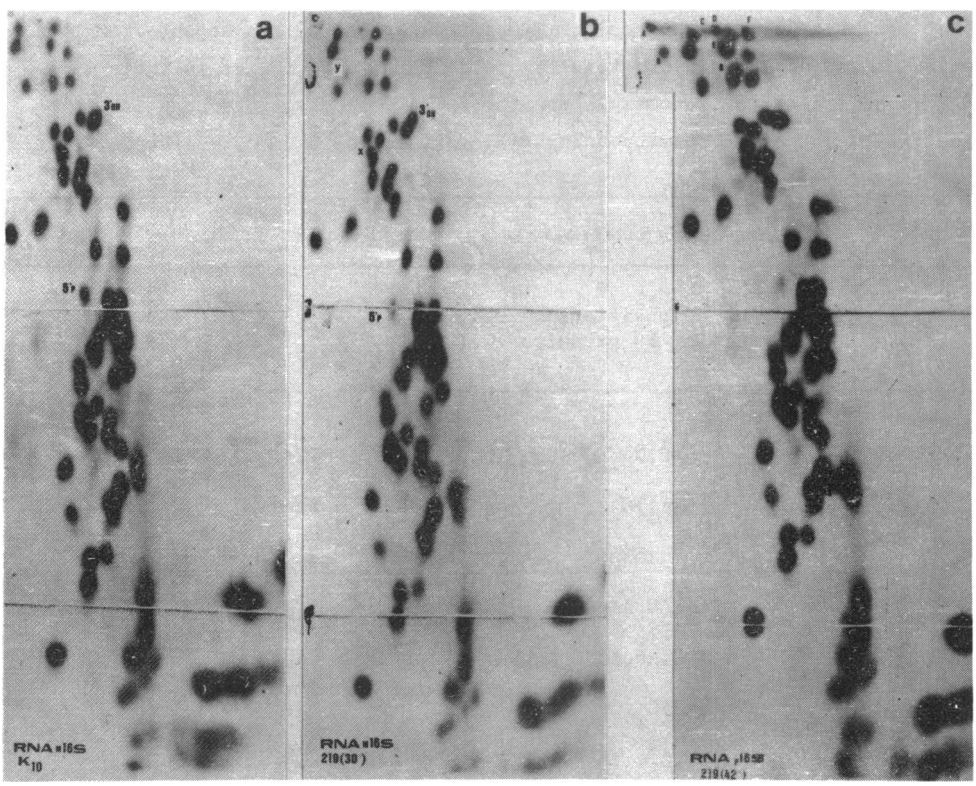

Fig. 1. Fingerprints of T_1 ribonuclease digests of m16S and p16S RNAs.
The RNAs were digested with T_1 ribonuclease and bacterial alkaline phosphatase and fingerprinted by electrophoresis on cellulose acetate (first dimension) and DEAE-paper (second dimension) as previously described[10]. Electrophoresis in the second dimension was continued until the blue marker[10] was about 70 cm from the origin.

RESULTS AND DISCUSSION

We initially examined the p16S RNA accumulated by strain 219 at 42°, to determine its relationship to the p16S RNA from a cold-sensitive mutant and from chloramphenicol-treated cells previously characterised by Lowry and Dahlberg[4]. The fingerprint of this RNA is shown in fig. 1(c). A fingerprint of m16S RNA from a wild-type strain, K_{10}, is also shown (fig.1(a)) for comparison. It may be seen that a number of additional spots are present on the fingerprint of the p16S RNA. These oligonucleotides were characterised by complete digestion with pancreatic ribonuclease. The products which were obtained are in close agreement with the results of Lowry and Dahlberg[4], and indicate that the p16S RNA accumulated by strain 219 is virtually identical with the p16S RNA species isolated by these authors under different conditions. We also find that the methylated sequences CCm^{7}GCG and m$^6_2$Am$^6_2$ACCUG are absent from the p16S RNA fingerprints. Our results are compared with theirs in table 1, and we have used their nomenclature for the oligonucleotides both in the table and on the corresponding fingerprint. We have noted three

Table 1. Additional oligonucleotides in p16S RNA of E. coli.

spot	compositions strain 219 products	compositions reported by Lowry and Dahlberg[4]
A	(AAU,U_{6-8},C)G	idem
B	(C,U_5)G	idem
C	absent	(AAAAU,AAAC,C,U_5)G
D	(AAAAU,AAAC,AU_{1-2},C_3,U_6)G	idem
E	(AAU,AU,AC,C_{1-2},U_3)G	idem
F	(AU,AC_2,C_{5-6},U_{5-6})AAAG	idem
G	(AU,C,U_3)AAG	(AAC,AU,C,U_3)G
H	AAAAAUG	AAAAUG
I	(AAU,AC,C)AAG	idem
a	pUG	idem

minor discrepancies between our findings and the published results of these authors: (i) Oligonucleotide C is practically absent from our fingerprints; its composition as reported by Lowry and Dahlberg[4] suggests it may arise by oversplitting of spot D during the T_1 ribonuclease digestion. (ii) We find oligonucleotide H to be AAAAAUG, agreeing with the revised sequence of Lowry and Dahlberg (J. Dahlberg, personal communication). (iii) Our oligonucleotide G contains an AAG sequence rather than AAC. We suspect that this might be a real strain difference.

We also examined the p16S RNA of a cold-sensitive sister strain, 217. The fingerprint (not shown here) and compositions of the additional oligonucleotides were identical to those of the p16S RNA from strain 219.

As a control experiment, we also fingerprinted the m16S RNA synthesised in strain 219 grown at the permissive temperature (34°). We found unexpectedly that the fingerprint differed from that of the m16S RNAs from the strains K_{10} (fig. 1(a)), 217 (not shown) or MRE 600[13]. While the 3'-terminal oligonucleotide is present in normal amounts, the 5'-terminal oligonucleotide usually found in m16S RNA digests is almost entirely absent (fig. 1(b)). However, two supplementary oligonucleotides, X and Y , which are absent from fingerprints of the m16S RNA from the wild-type strains or 217, may be seen on fingerprints of the m16S RNA from strain 219.

Complete hydrolysis of these oligonucleotides with pancreatic ribonuclease yielded the products AAAU + 2U from spot X, and AAAU + 3U from spot Y. The normal 5'-terminal oligonucleotide of the m16S RNA is pAAAUUG[13], and the dephosphorylated form of this oligonucleotide present on these fingerprints would yield AAAU + 1U upon pancreatic ribonuclease digestion. These findings suggest that the presence of spots X and Y, which together are found in roughly molar amounts, is related to the absence of the usual 5'-terminal oligonucleotide from fingerprints of the m16S RNA from strain 219. It is likely that the two supplementary spots arise from incompletely trimmed forms of the m16S RNA, in which either one or two additional U residues remain at the 5'-terminus of the 16S RNA molecule. However, it should be emphasised that it has not been formally established that the sequences of the oligonucleotides X and Y areUAAAUUG and UUAAAUUG respectively, and since these oligonucleotides were isolated from digests carried out in the presence of bacterial alkaline phosphatase it is not known whether they contain 5'-terminal phosphate moieties in the intact molecule.

The sud_2 mutation seems to be directly responsible for the presence of these oligonucleotides, since they are absent both from wild-type strains and from the cold-sensitive sister strain 217. If these oligonucleotides are indeed derived from m16S RNA species containing one or two additional U residues at the 5'-terminus, as seems probable, it is unclear whether these species are late intermediates in the maturation process which are accumulated because the final trimming steps cannot take place, or take place very slowly, or alternatively whether there is a mis-trimming of the RNA. Either of these explanations requires that the modification of protein S4, due to the sud_2 mutation, causes an alteration of the conformation of the trimming site affecting its recognition by the nucleolytic enzyme responsible for the maturation of the RNA. This could be brought about by the altered region of the protein being itself a part of the trimming site, or by the alteration causing a conformational change in an adjacent region of the p16S RNA or another protein lying within the site. In any event it is clear that the protein S4 plays an important role in maintaining the correct conformation of this site in precursor particles. In this strain the same modification in the protein S4 is responsible both for the incomplete maturation of the RNA synthesised at a permissive temperature, and for the complete block in ribosomal assembly, leading to the accumulation of 26S precursor particles, at a non-permissive temperature. It is not clear whether the complete block in the maturation process at 42° is due to the trimming site being altered to a greater extent at this higher temperature, preventing the trimming entirely, or whether the thermosensitivity is due to some other affect of this mutation becoming operative at 42°, independent of the trimming process.

Analogous precursor species, containing one or two additional U residues at their 5'-termini, have been described for the 5S RNA of E. coli[14], and are thought to be normally occurring intermediates in the maturation of this RNA. If the 16S RNA precursors described above also function as normal intermediates, then the properties of strain 219 could be explained in the following way. At the permissive temperature, the main maturation steps can occur, but the final trimming of the last extra residues at the 5'-terminus is slowed, and the late precursors are accumulated. The presence of these precursors in the 30S subunits does not seem to alter their biological activity,

and similar findings were obtained with regard to the late 5S RNA precursors which were present in functional 50S subunits[14]. At the non-permissive temperature, the trimming site might be altered to a greater degree, preventing all steps of the RNA maturation from being carried out.

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