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Escherichia coli 23S ribosomal RNA truncated at its 5' terminus

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ABSTRACT

In a strain of E. coli deficient in RNase III (ABL1), 23S rRNA has been shown to be present in incompletely processed form with extra nucleotides at both the 5' and 3' ends (King et al., 1984, Proc. Natl. Acad. Sci. U.S. 81, 185-188). RNA molecules with four different termini at the 5'end are observed in vivo, and are all found in polysomes. The shortest of these ("C3") is four nucleotides shorter than the accepted mature terminus. In growing cells of both wild-type and mutant strains up to 10% of the 23S rRNA chains contain the 5' C3 terminus. In stationary phase cells, the proportion of C3 termini remains the same in the wild-type cells; but C3 becomes the dominant terminus in the mutant. Species C3 is also one of the 5' termini of 23S rRNA generated in vitro from larger precursors by the action of purified RNase III. We therefore suggest that some form of RNase III may still exist in the mutant; and since no cleavage is detectable at any other RNase III-specific site, the remaining enzyme would have a particular affinity for the C3 cleavage site, especially in stationary phase cells. We raise the question whether the C3 terminus has a special role in cellular metabolism.

INTRODUCTION

In Escherichia coli, RNase III is known to initiate the processing of the primary transcript of rRNA to produce precursor 23S, 16S and 5S rRNAs which are further processed by other activities to yield mature rRNA species. We have reported earlier (1) that the RNase III-deficient mutant of E. coli (strain ABL1; ref.2) contains no mature 23S rRNA. At both its 5'and 3' ends species up to 100 nucleotides longer than the normal mature rRNA were found. At the 5' end four species were found. These included rRNA molecules with 20, 48 and 97 extra nucleotides. However, the fourth species noted, C3, was unexpectedly found to be four nucleotides shorter than the 5' terminus of the bulk 23S RNA in a wild-type strain D10 (RNase I^-; ref. 3). The unexpected shortness of this rRNA has led us to study its levels and origin more extensively, and to ask if it is functional in the cell.

METHODS

Preparation of polysomes and polysomal RNA

For the preparation of polysomes *E. coli* strain ABL1 or D10 was grown in 1 liter of Luria broth containing ^{3}H-uridine (0.5/mCi) to an absorbance of 0.5 at 550 nm. The cell cultures were quickly chilled over crushed ice in the presence of 100 µg/ml chloramphenicol and harvested in the cold by centrifugation at 3000 x g for 15 min. The cells were washed once with buffer A (10mM Tris-HCl pH 7.4, 2mM $CaCl_2$ and 5mM $MgCl_2$) and recentrifuged. The cell pellet was dispersed in 0.4 ml of lysozyme solution (0.12M Tris-HCl pH 7.8, 4mM EDTA and 20 mg/ml lysozyme) and incubated at 10°C for 10 min. One ml of the lysis solution (6 mM Tris-HCl pH 7.8, 35mM magnesium acetate, 90mM NH_4Cl, 1.5% Brij 35 and 0.1% sodium deoxycholate), also at 10°C, was added to it and incubation continued at 10°C until a clear lysate could be visually observed. A solution of RNase-free DNase I was added to a final concentration of 50 µg/ml and incubation continued for 5 min. The whole lysate was then centrifuged in the cold at 12000 x g for 3 min and the supernatant set aside. The pellet was resuspended in 0.5 ml of the lysis solution and centrifuged again as above. The supernatant was pooled with the first supernatant and cleared of any remaining cell debris by further centrifugation in the cold at 5000 x g for 10 min. 1.5 ml of the supernatant was then centrifuged at 4°C through a 38 ml linear gradient of 10-30% sucrose in 10mM Tris-HCl pH 7.8, containing 1M NH_4Cl, 40 mM magnesium acetate, 2 mM EDTA and 10 mM ß-mercaptoethanol, at 30,000 xg for 14 h. Previously purified 70S ribosomes and the ribosomal subunits from *E. coli* were centrifuged as markers in replicate gradients. Polysomes of different sizes were localized in gradient fractions by comparison to marker positions. The conversion of all polysomes to monosomes by RNase treatment was confirmed. Only the largest polysomes were analyzed for the termini of 23S rRNA.

Polysomal RNA was prepared from the gradient fractions by phenol extraction in the presence of 50 µg/ml of yeast tRNA as carrier and precipitation with ethanol. The RNA was then dissolved in hybridization buffer as described in ref. 1 (100 µl for each sample).

Isolation of total RNA from cells at different phases of growth.

E. coli strains (D10 or ABL1) were grown in Luria broth from an absorption at 550 nm of 0.05/ml to 1.6/ml. Portions of the growing cultures were withdrawn at different stages of the growth cycle to yield approximately equal number of cells and quickly chilled over crushed ice. Cells were collected by centrifugation in the cold and cell pellets rinsed with buffer A. The cells

were suspended and lysed in RNA isolation buffer (0.2M sodium acetate pH 5.5, 0.1 M NaCl, 1.0 mM EDTA and 0.5% SDS). Total RNA was then obtained by phenol extraction and precipitation with cold ethanol. The respective RNA precipitates were dissolved in hybridization buffer and solutions brought to uniform concentration before hybridization to the DNA probes.

S1 nuclease mapping

The single stranded DNA probe for the 5' end of 23S rRNA used for S1 nuclease mapping was as described in (4), 331 nucleotides long and complementary to 107 nucleotides of mature 23S rRNA sequence and 96 nucleotides of adjacent 5' precursor rRNA sequence [nucleotides 3404-3606 of the rrnB sequence (5)]; an additional 129 nucleotides of pBR322 sequence were at its 3' end. The details of hybridization of 23S rRNA with the DNA probe, S1 nuclease digestion of the hybrids, and analysis of the protected regions by denaturing polyacrylamide gel electrophoresis are given in (4). The concentration of polyacrylamide gels used in different experiments are given in legends to figures. Estimates of the relative amounts of RNA species were obtained by cutting slices containing individual RNA species out of a gel and counting them by Cerenkov radiation in a Packard liquid scintillation counter.

RNase III reaction and analysis of products

Five µg of 50 S ribosomes from strain ABL1 (prepared as described in ref.1) were incubated with or without purified RNase III (a gift from John Dunn, Brookhaven National Laboratory) in 50 µL of 10mM Tris-HCl pH 7.4 containing 5mM $MgCl_2$, 100mM NH_4Cl and 1mM β-mercaptoethanol, at 37°C for 1 h. Total RNA was phenol-extracted from the incubation mixture in the presence of 20 µg of yeast tRNA as carrier and precipitated with ethanol. The RNA precipitate was dissolved in hybridization buffer and analysed by S1 nuclease mapping as above.

RESULTS

Figure 1 shows S1 nuclease analyses of the 5' end of 23S rRNA; C3 and longer species are found in wild-type strain D10 as well as in strain ABL1. Both the mature and C3 species were found in polysomes of growing and stationary phase D10 cells; longer precursors were seen only in the actively growing cells (Fig. 2, lanes 1 and 2).

Although the C3 species usually accounts for no more than 10% of the 5' termini of 23S rRNA in growing mutant cells, it increases sharply in amount in mutant cells that are incubated into the stationary phase of growth. The increase is extreme, until the C3 species accounts for the 5' termini of all

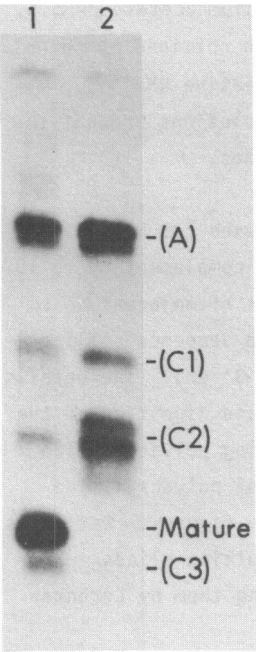

Fig. 1. S1 nuclease analysis of the 5' termini of total cellular 23S rRNA. Total 23S rRNA isolated from wild-type (D10) and RNase III-deficient (ABL1) strains of E. coli was isolated, hybridized to a single-stranded DNA probe complementary to sequences at the 5' end of 23S rRNA and digested with S1 nuclease as described in Methods. The protected hybrid molecules were denatured by boiling in 50% formamide and analysed on a 10% polyacrylamide gel. The autoradiogram shows the D10 and ABL1 RNAs in lanes 1 and 2, respectively. The positions of 23S rRNA species with different 5' termini (A, C1, C2 and C3, and mature as in ref. 1) are indicated.

the 23S rRNA in polysomes (Fig.2, lane 4), and for 40% of the 5' termini in total cellular RNA. As Fig. 3 shows, the amount drops in growing cells and increases sharply again as cells enter the stationary phase of growth.

Since species C3 is found in polysomes (Fig. 2), one might anticipate that it is at least partially functional. In support of this possibility, cells inoculated from stationary phase culture (with all the 5' termini in polysomes as C3) grow rapidly in enriched broth -- and without any detectable lag (data not shown).

The origin of species C3 was unexpectedly clarified when 50S ribosomes from strain ABL1 were incubated with highly purified RNase III (Fig. 4). Earlier studies with primary transcripts ("30S pre-rRNA") isolated from E. coli showed that cleavage occurs at one site 7 nucleotides from the mature 5' end of 23S rRNA (Fig. 5), with some suggestion of further cleavages that were not specified (6). When the S1 nuclease assay was applied to the products of the reactions with 50S ribosomes, the RNA chains with long precursor sequences at their 5'ends (lane 1 in Fig. 4) were found to have been cleaved at three sites (lane 3, Fig. 4). Two of these cleavage points yield precursors of the accepted mature rRNA. [One is the species 7 nucleotides longer characterized earlier (6); the other is three nucleotides longer than mature rRNA

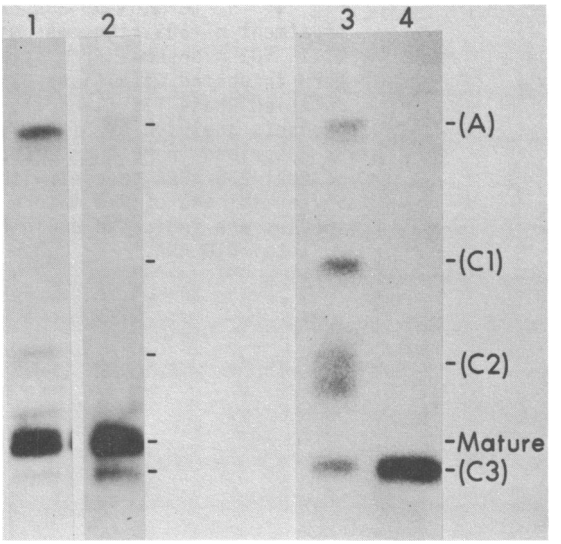

Fig. 2. S1 nuclease analysis of the 5' termini of 23S rRNA present in polysomes from strains D10 and ABL1. Polysomal fractions from wild-type and mutant E. coli were prepared and RNA obtained as in Methods. The RNA was then analysed as in Fig. 1. The autoradiogram shows results with polysomal RNA from strain D10 (lanes 1 and 2) and from ABL (lanes 3 and 4); Lanes 1 and 3, polysomal RNA from growing cells; lanes 2 and 4, polysomal RNA from stationary phase cells. The positions of various species are indicated as in Fig. 1.

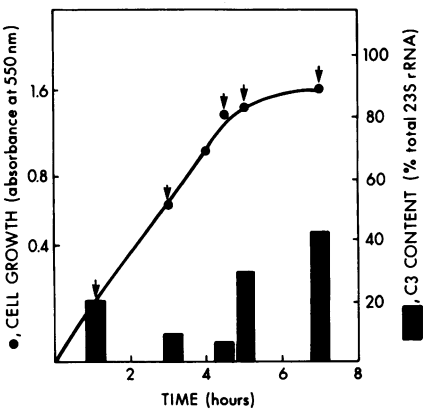

Fig. 3. Relative amounts of the C3 5' terminus of 23S rRNA present in strain ABL1 at different phases of growth. Total cellular RNA was prepared from ABL1 cells at different stages of growth, and S1 nuclease analysis performed as in Fig. 1. The relative amounts of various 23S rRNA species were determined as described in Methods. Bars represent the percentage of C3 species in total RNA preparations from cells at the growth times indicated by arrows.

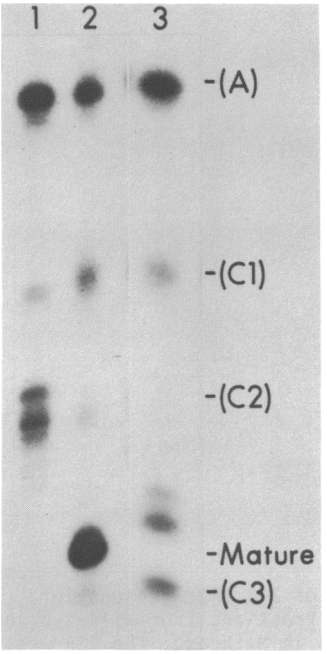

Fig. 4. 5' termini of 23S rRNA after treatment of 50S ribosomes with RNase III. 50S ribosomes from strain ABL1 were incubated with (lane 3) or without purified RNase III (lane 1), and the products analysed by S1 nuclease mapping as described in Methods. The positions of ABL1 23S rRNA species with different 5' termini and of D10 mature 23S rRNA species are indicated as in Fig. 1. Lane 2, total D10 RNA.

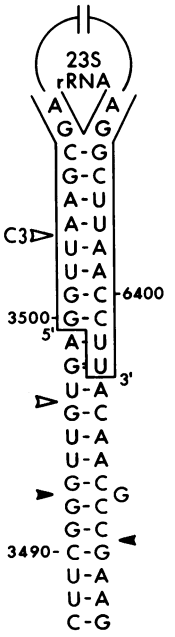

Fig. 5. The nucleotide sequence adjoining the 5' and 3' ends of 23S rRNA, shown in the secondary structure suggested by refs. 5 and 6. RNase III cleavages reported in the primary transcript of rRNA (ref. 6) are indicated by closed arrowheads; other RNase III cleavage sites are indicated by open arrowheads.

and will be characterized elsewhere.] But the third species has the mobility characteristic of the C3 species. Thus, although little or no RNase III activity can be detected in the mutant strain, some termini characteristic of its action at one particular site *in vitro* are generated *in vivo*.

DISCUSSION

Since the mutant strain is viable, it was earlier concluded that at least some of the unprocessed 23S rRNA species must be functional in the cells, and that maturation may not be obligatory for the function of 50S ribosomes. The results presented here suggest that probably all of the rRNA species are functional. First, RNA molecules with all the various 5' termini are found in polysomes in the mutant and wild-type strain. Second, even the shortest form with the C3 terminus, which increases to 40 to 50% of the total 23S rRNA in the stationary phase, can support an optimal growth rate with virtually no lag when growth of the culture resumes.

Species C3 results from a cleavage in the double-stranded region of 23S rRNA, four nucleotides distal to the conventionally accepted mature terminus (Fig. 5). We observe production of species C3 in the reaction of purified RNase III with 50S ribosomes from strain ABL1 (Fig. 4, lane 3). This suggests that some RNase III may still be present in the deficient strain, in a form that produces C3 but does not cleave elsewhere to initiate normal processing reactions at a detectable rate. The modified enzyme apparently not only shows a preference for the C3 site, but also cleaves at that site more efficiently in stationary phase cells, where the amounts of C3 increase at the expense of species with longer precursor sequences. Perhaps the C3 site is more exposed in ribosomes during the stationary phase, or RNase III is activated in stationary phase cells (cf. ref. 7). The phenomenon appears to be limited to the 5' end of the rRNA, since no comparable termini shorter than the accepted 3' end have been detected or generated in ribosomes from these cells at any stage of growth (work in progress).

Species C3 also occurs in significant amounts in wild-type cells; but in contrast to the case in the mutant, in wild-type cells, normal processing occurs so rapidly (4) that most RNA chains quickly reach the state with essentially mature termini. The small base-paired stem of only 8 nucleotides remaining in mature 23 S rRNA (Fig. 5) is presumably not long enough to be a further substrate for RNase III. Thus, in wild-type cells, small and somewhat variable amounts of species C3 can arise only during the brief interval when the longer stems are transiently present in pre-rRNA.

The puzzle remains whether heterogeneity at the 5'end of 23S rRNA serves any function in cells. On the one hand, it is now clear that cells are very permissive in their requirements for the termini of 23S rRNA -- molecules 4 nucleotides shorter or 100 nucleotides longer at their 5' end are apparently functional. In that case, species C3 could be simply an accident that can occur and is of no consequence for cellular survival. On the other hand, cleavage to form a species shorter than the normal terminus might be maintained evolutionarily if heterogeneity of the species played some role. Calculations show that the mature 23S rRNA could still contain a putative d.s. stem 8 nucleotides long, but the cleavage at C3 could destabilize that base-paired region. Such a ribosome could have an altered conformation, which might affect the differential translation of mRNAs in the mutant or even in wild-type cells. It may be relevant that the translation of various mRNA species is inhibited or promoted in strain ABL1 (8-10).

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