# STRUCTURE OF A 30 S PRE-RIBOSOMAL RNA OF *E. COLI*

F. HAYES and M. VASSEUR

*Institut de biologie physico-chimique, 13, rue Pierre et Marie Curie, 75005 Paris, France*

N. NIKOLAEV and D. SCHLESSINGER

*Department of Microbiology, Washington University School of Medicine, St Louis Mo. 63110 – USA*

J. SRI WIDADA, A. KROL and C. BRANLANT

*Laboratoire de Bio-Chimie, Institut de Biologie Moléculaire et Cellulaire du CNRS, 67 Strasbourg, France*

## 1. Introduction

The primary product of transcription of the ribosomal RNA genes of *E. coli* is long polyribonucleotide chain with a sedimentation coefficient of 30 S (30 S pre-rRNA) containing one complete sequence of 16 S rRNA and one of 23 S rRNA together with about 1300 nucleotides in additional sequences [1–3]. This large ribosomal precursor RNA has not been detected in wild type *E. coli* presumably because it is cleaved into smaller fragments before its transcription is completed but it has been isolated from an *E. coli* mutant (AB 301-105) which is deficient in RNase III activity. In vivo processing of the 30 S pre-rRNA leads to formation of 25 S and 17.5 S species, precursors of the 23 S and 16 S rRNAs. Accumulation of these intermediates precursors is increased significantly if bacteria are incubated in the presence of chloramphenicol [4], and limited treatment of isolated 30 S pre-rRNA in vitro with purified RNase III produces large products sedimenting at 25 S and 17.5 S plus several additional smaller fragments [4,5]. Certain aspects of the primary sequences of 30 S pre-rRNA and of the products of its in vivo and in vitro processing are reported here.

## 2. Materials and methods

30 S pre-rRNA and its 25 S and 17.5 S in vivo cleavage products were isolated by sucrose gradient sedimentation as previously described from *E. coli* AB 105 labelled with $^{32}$P in the presence [4] or absence [1] of chloramphenicol.

RNase III was prepared as before, the products of its action on isolated 30 S pre-rRNA were fractionated on sucrose gradients, and the purity of isolated fractions was detected by acrylamide gel electrophoresis [4,5].

Isolated $^{32}$P-labelled RNA fractions were analysed by; (1) $T_1$ RNase-alkaline phosphatase fingerprinting [6]; (2) Determination of 5' terminal nucleoside triphosphates by two-dimensional electrophoresis of alkaline hydrolysates [7] and proof of their structures by digestion with snake venom phosphodiesterase and isolation of nucleoside diphosphates and inorganic pyrophosphate [8]; (3) Determination in one case of the 5' terminal RNase $T_1$ cleaved oligonucleotide by two-dimensional 'diagonal' electrophoresis [7].

## 3. Results and discussion

### 3.1. $T_1$ RNase fingerprints

Fig. 1 shows the fingerprint of 17.5 S rRNA precursor isolated from $^{32}$P-labelled chloramphenicol-treated *E. coli* AB105. Fingerprints of 17.5 S pre-rRNA isolated from cells labelled in the absence of chloramphenicol or from the products of in vitro cleavage of 30 S pre-rRNA by purified RNase III are essentially the same. In spite of a slight contamination by degraded
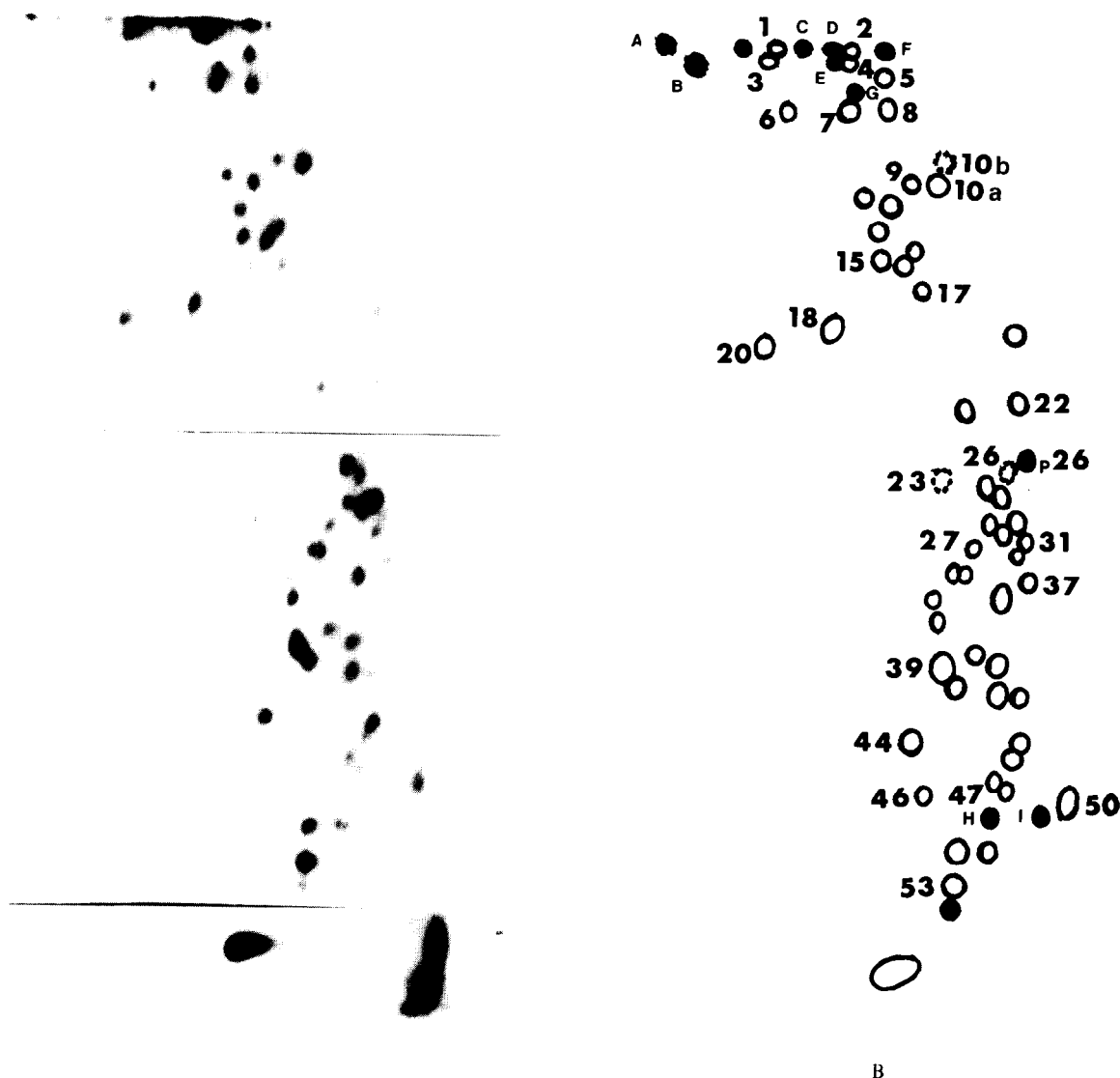
Fig.1. Fingerprint of 17.5 S pre rRNA of *E. coli* AB 105. 17.5 S pre-rRNA isolated from [32] P-labelled cells was digested with T1 RNase plus alkaline phosphatase and the fingerprint was obtained as usual except that migration in the second dimension was continued until the blue marker had migrated 68 cm. Numbered and unnumbered open spots in fig.1B are oligonucleotides of mature 16 S rRNA; solid spots: are additional oligonucleotides.

25 S rRNA it is clear that in each case the fingerprint resembles those published for CM-16 S RNA [9] and for p16 S RNA accumulated during growth at 42°C by *E. coli* 219 a thermosensitive ribosomal assembly defective mutant [10] i.e. it contains about eight extra oligonucleotides (spots) absent in the fingerprint of *E. coli* 16 S rRNA, and shows only traces of the 3′

and 5′ terminal oligonucleotides of 16 S rRNA (spots 10b, and 23 in the nomenclature of Fellner et al., [11]. The absence of spot 26 which corresponds to the methylated oligonucleotide U–m⁵C–A–C–A–C–C–A–U–G found in mature 16 S rRNA [11] may also be noted. Comparison of the fingerprint of 17.5 S pre-rRNA with that of the p16 S rRNA of 27 S ribo-

Fig.2. (A,B). Fingerprint of 25 S pre rRNA of *E. coli* AB 105. 25 S pre-rRNA was prepared from [32] P-labelled cells and fingerprinted as above. Open and closed spots in fig. 2B have the same signification as in Fig. 1B. (C) Fingerprint of mature 23 S RNA.

some precursor particles [12] shows clearly that the latter species is considerably shorter than the former containing only four of the eight extra oligonucleotides found in the 17.5 S pre-rRNA.

Fig.2B shows the fingerprint of 25 S pre-rRNA isolated from *E. coli* A105 labelled for a short time, in the absence of chloramphenicol. This fingerprint contains all the spots found in that of mature 23 S rRNA (fig.2C), except that of the 3′OH oligonucleotide, which is virtually absent. It has been noticed that

the spot corresponding to the methylated oligonucleotide $m_{10}$ (in the nomenclature of Fellner [13]): $A-C-A-U-A-U-m^1G-\psi-T-G$ is present. The absence of methylation in this oligonucleotide would give a new $T_1$ product $A-C-A-U-A-U-G$; just a little amount of the corresponding spot is present. This suggests that this 25 S pre-rRNA is at least partially methylated. The fingerprint of 25 S pre-rRNA contains a large number of spots which are not present in that of mature 23 S rRNA, eight of which are

Fig. 3. (A) Fingerprint of a low mol. wt fragment released during RNase III treatment of 30 S pre-rRNA in vitro. (B) Fingerprint of 5 S rRNA of *E. coli* D10. The RNAs were digested with RNase T1 only.

clearly visible. Several of these additional spots are in a position where there is no spot in the fingerprint of 17.5 S pre-rRNA, inferring that they certainly do not derive from a contamination by 17.5 S RNA. Therefore they do correspond to extra sequences located at the extremities of mature 23 S RNA. It is remarkable that these additional spots contain a large number of uridylic and adenylic residues: spot 1 contains at least 6 U, spot 2: (5 U, A or C) G, spots 3 and 4 contain 5 U, spot 5: U–U–U–U–G, spot 6: (3 U, 2 A) G, spot 7: (2 U, 4 A) G and spot 8: (2 U, A) G. ^{32}P-labelled 23 S pre-rRNA was also isolated from *E. coli* AB 105 cells treated with chloramphenicol. It contains all of the extra spots described above and some other oligonucleotides. It appears to be more heterogenous since the molar ratio of the extra spots is variable.

Fig.3 compares the fingerprint of one of the small fragments produced during RNase III cleavage of 30 S pre-rRNA with that of 5 S rRNA of *E. coli* D10. In spite of the relatively impure state of the RNase III fragment the resemblance between the two fingerprints is striking; all the small and several of the large oligonucleotides of 5 S rRNA could be present in this fragment (spots 1–14, 20, 31). We conclude that the RNase III cleavage fragment analysed certainly contains the sequence of 5 S rRNA.

### 3.2. 5' Terminal triphosphate residues

Two-dimensional electrophoretic analyses of alkaline hydrolysates of 30 S pre-rRNA isolated from *E. coli* AB 105 labelled with ^{32}P in the presence of chloramphenicol led to isolation of a mixture of pppAp and pppGp (fig.4) from which after treatment with snake venom phosphodiesterase, high yields of pAp, pGp and ppi were recovered (table 1). These results show that 30 S pre-rRNA isolated from cells labelled in the presence of chloramphenicol carries a 5' terminal triphosphate group and that the 5' terminal nucleotide is either A or G (A : G ~ 3 : 2). A similar analysis of an alkaline hydrolysate of 30 S pre-rRNA labelled in the absence of chloramphenicol revealed the presence of about 0.5 mol/mole of a 5' triphosphate residue but the amount of radioactivity isolated (600 counts/10 min.) was insufficient to permit determination of the ratio of pppAp: pppGp.

The result of a two-dimensional diagonal electrophoretic analysis of a $T_1$ RNase-alkaline phosphatase
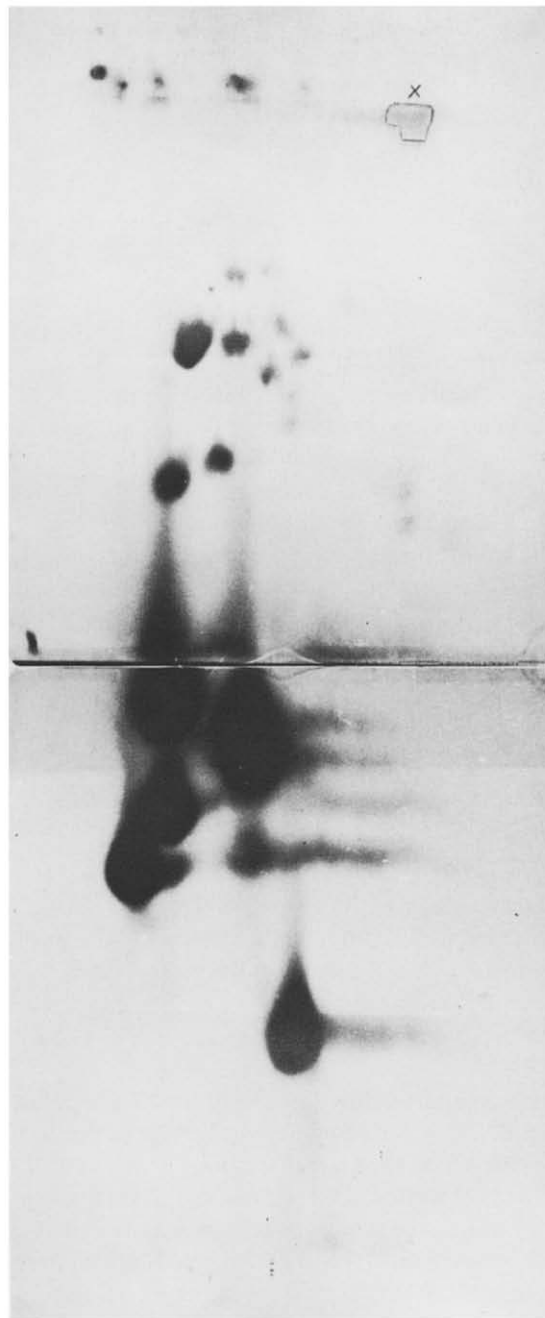


Fig.4. Two-dimensional electrophoresis of an alkaline digest of 30 S pre-rRNA. First dimension left to right, second dimension top to the bottom. The cross indicates the position of nucleoside tetra phosphates.

Table 1

| 30 S pre-rRNA sample | Total radioactivity isolated as pppxp cts/10 min | moles per 6600 nucleotides of 30 S pre-rRNA | | | |
|---|---|---|---|---|---|
| | | pppXp | pAp | pGp | ppi |
| 1 | 4000 | 1.1–1.2 | 0.6 | 0.4 | 0.85 |
| 2 | 20 000 | 1.2 | 0.8 | 0.4 | – |
| | 14 000 | 1.2 | 0.7 | 0.5 | 0.7 |

hydrolysate of 30 S pre-rRNA from cells labelled with $^{32}$P in the presence of chloramphenicol is shown in fig.5. A faint spot with a very low mobility in the first and a very high mobility in the second dimension (arrow fig.5) is presumed to correspond to the 5′ terminal oligonucleotide of the 30 S pre-rRNA. Analysis of the products of alkaline hydrolysis of this spot revealed the presence of equimolar amounts of A, U and C. We therefore conclude that the sequence pppA (U,C) Gp . . . . is present at the 5′ terminus of a fraction of 30 S pre-rRNA. A 5′ terminal pppGp would of course not be detected by the procedure used here.

## 4. Conclusions

The results presented here show that 30 S pre-rRNA isolated from CM treated cells contains the primary sequence of 23 S and 16 S rRNA and very likely that of 5 S rRNA and strongly suggest that this precursor species (normal and CM treated cells) carries a 5′ terminal triphosphate residue and is therefore the primary product of transcription of rRNA genes.

However preliminary studies of the 17.5 S and 15 S RNAs produced by in vivo cleavage of the 30 S pre-rRNA have shown that alkaline hydrolysis of these RNAs (CM treated cells) releases significant amounts of adenosine and guanosine tetraphosphate (0.1–0.5 mol per mole of 17.5 S and 25 S pre-rRNA, A: ≃ 3: 2). In contrast similar analyses of the 17.5 S and 25 S products of RNase III treatment of 30 S pre-rRNA in vitro and of residual 30 S material recovered from RNase III digestion mixtures shows that none of them contains a 5′ terminal triphosphate residue. The presence of 5′ triphosphate residues in the products of in



Fig.5. Phosphatase diagonal of a T1 RNase digest of 30 S pre-rRNA. First dimension left to right, second dimension top to bottom. The arrow indicates the 5′terminal oligonucleotide of the RNA.

vivo cleavage of 30 S pre-rRNA is unexpected and as yet unexplained. It may reflect some independant transcription of 17.5 S and 25 S RNA species. Alternatively, if it is due to a post-synthetic modification of the 5′ termini of these RNAs, or to the presence of contaminating triphosphate terminated polynucleotides in the isolated products it is evident that the significance of observation that pppAp and pppGp are released during alkaline hydrolysis of 30 S pre-rRNA must be reevaluated. Further experiments will be required to clarify this point.

The 17.5 S pre-rRNA produced by in vivo cleavage of 30 S pre-rRNA is very similar to CM-16 S RNA and to p16 S RNA accumulated during growth at 42°C by *E. coli* 219. The 25 S rRNA is larger than the p23 S rRNA of 43 S precursor of the 50 S ribosomal subunit of *E. coli* (unpublished results). The additional oligonucleotides contain a large amount of urydilic and adenylic acid residues. The presence of clusters of urydilic acids in the regions of pre-rRNA not contained in mature rRNA seems to be a general feature. In the case of 17.5 S pre-rRNA most of the detected additional oligonucleotides contain more than 5 U [9,10]. These clusters of urydilic acids have perhaps a role in the processing of maturation. Exhaustive attack on 25 S rRNA by RNase III removes more of the extra sequences to produce a final product very similar to the mature 23 S rRNA ([5] and work in progress). RNase III may have a specificity for double stranded RNA regions containing a large number of A–U base pairing.

After this work had been carried out we learned that Steitz and Ginsburg [14] had independently obtained results in close agreement with ours.

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