IN VITRO MATURATION OF A 16 S RNA PRECURSOR

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1. Introduction

We have previously shown [1] that the major precursor of the *E. coli* 30S ribosome, a ribonucleoprotein particle of sedimentation coefficient '27S' contains a 16S RNA precursor (p16rRNA) of sedimentation coefficient '17S'. This p16rRNA has a lower electrophoretic mobility and contains a longer polynucleotidic chain than the corresponding mature species (m16rRNA). It is known that the 3′ and 5′ extremities are different in the two species but the distribution of the extra nucleotides at the extremities of the p16rRNA is unknown.

2. Materials and methods

In the present study we examine the modification of p16rRNA produced by incubation of the 27S particle with an S30 extract of *E. coli*. Polyacrylamide gel electrophoresis of the RNA extracted from the incubation mixture at various times shows that the p16rRNA is progressively converted to a species which possesses the same electrophoretic mobility as m16rRNA (fig. 1, panels B,D,F). This transformation is not accompanied by significant degradation of p16rRNA since the RNA contents of equal aliquots of the incubation mixture are analyzed on gels B,D, and F (fig. 1) and, as can be seen, an approximately constant amount of tritium labelled RNA is found in the p16rRNA–m16rRNA regions of these gels. If the S30 content of incubation mixtures is replaced by an equal volume of buffer extensive degradation of p16rRNA takes place and no accumulation of material with the electrophoretic properties of m16rRNA is observed (fig. 1, panels A,C,E. RNA samples isolated from equal aliquots of an incubation mixture). The small amount of tritiated RNA with the same electrophoretic

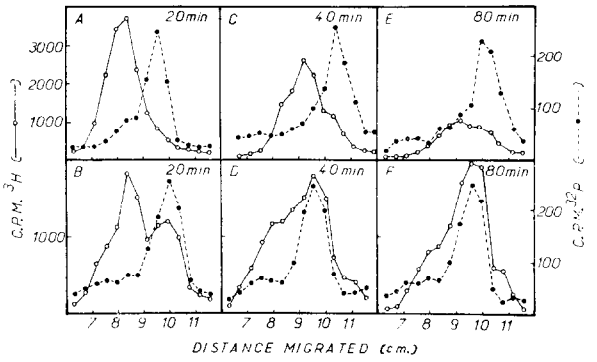

Fig. 1. Polyacrylamide gel electrophoresis of in vitro maturation products. ^{3}H-Labelled 27S precursor particles were isolated by sucrose gradient sedimentation of extracts of [^{3}H]uracil pulse labelled exponentially growing *E. coli* D10 as described previously [2]. Samples of the 27S peak fractions of the sucrose gradients were used without further treatment. ^{32}P-labelled m16rRNA was prepared from *E. coli* D10 grown for several generations in the presence of [^{32}P] orthophosphate. In vitro incubation mixtures contained the following components in a final vol of 200 μl. (A,C,E): 100 μl of sucrose gradient 27S peak fractions containing ^{3}H-labelled 27S particles 0.005 M Tris–HCl, pH 7.4, 0.01 M $MgCl_2$, and approximately 15% sucrose (w/v), 300 μl of TMKSH buffer (0.005 M Tris–HCl, pH 7.4; 0.01 M $MgCl_2$; 0.05 M KCl; 0.006 M β-mercaptoethanol). (B,D,F): 100 μl of the suspension of ^{3}H-labelled 27S particles and 300 μl of an S30 extract of *E. coli* D10 prepared in TMKSH. Mixtures were incubated at 37°C and samples of 100 μl were removed after 20, 40, and 80 min incubation. RNA was prepared from each sample by phenol extraction, mixed with ^{32}P m16rRNA and analysed on 13 cm, 4% polyacrylamide gels according to Loening [6]. After electrophoresis gels were frozen, and cut into 3 mm slices which were incubated overnight at room temperature with 0.5 ml of concentrated NH_4OH before counting in a toluene based Triton X 100-PPO-POPOP scintillation fluid. Zero time controls (± S30), not shown in fig. 1, gave results identical with those shown in panel A: (——○——) [^{3}H]uracil labelled p16rRNA and its in vitro transformation products; (——●——) ^{32}P-labelled m16rRNA.

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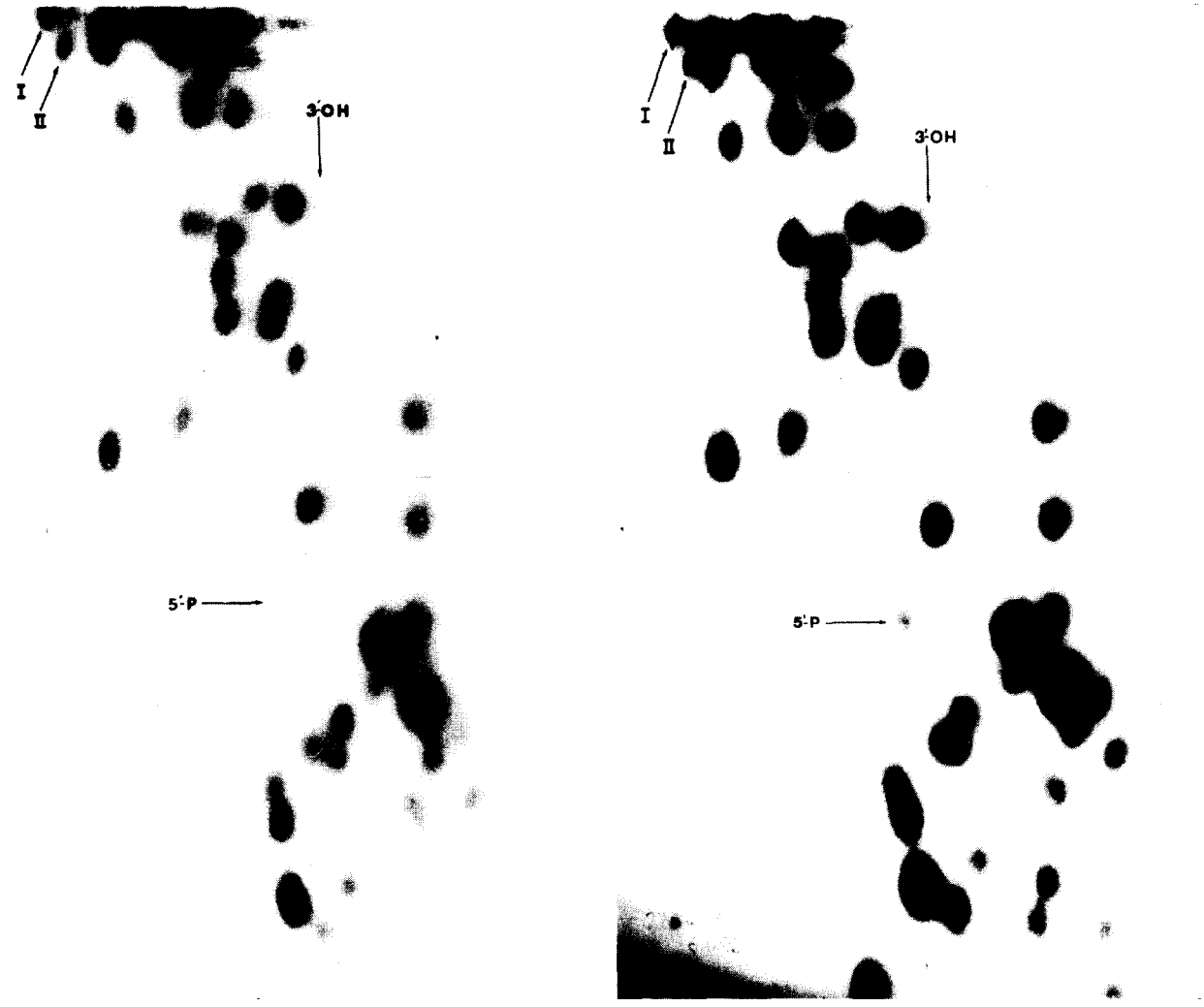

Fig. 2. Fingerprints of p16rRNA before and after in vitro maturation were obtained by the procedure of Sanger et al. [3].

mobility as the ^{32}P-labelled m16rRNA in panels C and E of fig. 1 is probably present in this form in the preparation of 27S precursor particles since we have observed both in earlier work [2] and in the present study that fingerprints of p16rRNA isolated from 27S particles usually contain small amounts of the terminal oligonucleotides of m16rRNA.

3. Results and discussion

It can be concluded from these experiments that in vitro conversion of p16rRNA to a product with the electrophoretic mobility of m16rRNA requires the presence of one or more components of an S30 bacterial extract. Attempts to isolate and characterize these components are in progress.

In order to verify that the change in the electrophoretic mobility of their RNA component produced by the incubation of 27S precursor particles in an S30 extract in vitro is due to a reduction in the length of the p16rRNA molecule RNAs were extracted from ^{32}P-labelled 27S particles before and after in vitro incubation, purified on sucrose gradients and analysed by fin-

gerprinting after hydrolysis with T_1 RNAse and alkaline phosphatase according to the method of Sanger et al. [3]. Fingerprints of p16rRNA before and after in vitro maturation are shown in fig. 2. In each case, 80–90% of the RNA added to the in vitro incubation system in the form of 27S particles was recovered in the 16–17S region of the sucrose gradients used to purify the reaction products confirming our earlier observation that only very limited degradation of the p16rRNA to small fragments takes place under the incubation conditions used. The relative amounts of mature 5′-P and 3′-OH termini and of extra oligonucleotides I and II [2] in fingerprints of RNA extracted from 27S particles before and after maturation were then measured. Extra nucleotides III and IV [2] were not studied because they are not well separated from other spots in the fingerprints. Table 1 summarizes the results obtained in three experiments of this kind. In the course of this work we have also observed that the oligonucleotide corresponding to the sequence m_2^6 A m_2^6 A CCUG which is known to be present in the m16rRNA and absent in p16rRNA [2,5] does not appear after incubation of 27S particles with an S30 extract. The maturation that we are observing therefore does not seem to be accompanied by methylation of p16rRNA at least in the case of this oligonucleotide.

Table 1
Stoichiometry of in vitro maturation of p16rRNA

Exp.	Oligo-nucleotide	Molarity		Percent maturation
		Before maturation	After maturation	
1	5′-terminal	0.13	0.40	31
	3′-terminal	0	0.16	16
	I	0.60	0.33	45
	II	0.70	0.16	77
2	5′-terminal	0.05	0.06	1
	3′-terminal	0	0	0
	I	0.73	0.30	59
	II	0.75	0.33	56
3	5′-terminal	0.34	0.76	65
	3′-terminal	0.30	0.45	21
	I	0.56	0.36	36
	II	0.55	0.23	58

Spots corresponding to the 5′-P terminal oligonucleotide of m16rRNA, to extra oligonucleotides I and II of p16rRNA, and to several oligonucleotides present in known molar amounts in p16rRNA and m16rRNA were cut out of fingerprints of the precursor RNA and of its in vitro maturation products, and their ^{32}P contents determined. The molar amounts of terminal and extra oligonucleotides were then calculated. In the case of the 3′-OH terminal oligonucleotide the molar amount was obtained by measuring the total radioactivity in spots 10a–10b which were cut out together and substracting from this quantity the calculated amount of radioactivity for a molar content of oligonucleotide 10a. Figures given in the right hand column of table express the increase or decrease in amount of each oligonucleotide as a percentage of the increase or decrease expected for total maturation of the p16rRNA.

Inspection of the results in table 1 shows that they vary considerably from one experiment to the next. This is not surprising since the system of maturation used here is not yet very well defined. In addition, in the same experiment the disappearance of the extra oligonucleotides I and II and the appearance of mature terminal sequences is not coordinated. This is particularly notable in experiment 2 in which significant amounts of oligonucleotides I and II were lost without appearance of mature terminal sequences. Two factors may contribute to the diversity of the results obtained in our experiments. The fact that maturation of the two ends of the p16rRNA molecule seems to be independent (exps. 1, 3 in table 1) can be explained by assuming that several enzymes are involved in the maturation process. We recall that Smith et al. in their study of the maturation of the precursor of tyrosine tRNA conclude that processing at the two ends of the molecule is carried out by different enzyme activities [4]. The different amounts of the 5′-P, and 3′OH terminal oligonucleotides of m16rRNA found in the preparations of 27S precursor particles all of which were homogeneous as judged by their sedimentation properties, and the non coordinate disappearance of oligonucleotides I and II and appearance of mature termini during the maturation process (compare exp.2, and exps1, 3 in table 1) suggest that precursor particle preparations are not homogeneous. Particles containing qualitatively different protein complements or RNAs with different conformations could present identical sedimentation properties but react differently with maturation enzymes.

These experiments lead to the conclusion that incubation of 27S ribosome precursor particles in an S30 extract causes trimming of their p16rRNA with partial or complete loss of its extra nucleotide content. The in vitro process seems to be at least partially specific since little fragmentation of the p16rRNA is seen and the 5′-P and 3′-OH terminal sequences of m16rRNA are generated.

However the in vitro conditions so far used achieve only partial maturation of p16rRNA as shown by the fact that generation of mature 5′-P and 3′-OH terminal sequences is incomplete and that methylation of the precursor RNA does not seem to occur. We are at present studying the purification of maturation activities from S30 extracts and the improvement of the quantitative assay of the oligonucleotides involved in the maturation process.

Acknowledgements

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