Processing of bacteriophage T4 tRNAs: a precursor of species 1 RNA

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A precursor molecule of species 1 RNA, p2Sp1, that accumulates when an *rne* (RNase E$^-$) mutant is infected with a T4 deletion mutant (Δ27) is also found after infection of an *rne* host mutant by different deletion mutants or wild type bacteriophage T4. Low levels of this molecule were also found in a wild-type host infected with a wild-type T4. This precursor molecule accumulates at higher concentrations at 43°C as compared to 30°C or 37°C. Structural analysis of the precursor molecules from the different sources has shown a complete identity of p2Sp1 RNA isolated from the different sources. Therefore, we suggest that this precursor is a normal intermediate in processing of T4 tRNAs, and that it is unrelated to a particular T4 deletion strain. Since RNase E does not process this precursor, its accumulation in an *rne* mutant reflects an interaction between RNase E and the enzyme that processes this intermediate.

Ribonuclease E rne *Gel electrophoresis* *T1 fingerprinting* *Deletion mutants* *RNA processing complex*

1. INTRODUCTION

Analysis of T4 RNA in an RNase E$^-$ mutant of *Escherichia coli* infected with T4Δ27, a deletion mutant of bacteriophage T4, revealed the accumulation of a precursor of species 1 RNA that was named p2Sp1 RNA [1]. The T4Δ27 contains an internal deletion that removes 7 of the 10 genes in the T4 tRNA cluster, leaving intact the first 2 and the last gene [1–3]. In this deletion the 3′-end of the second gene is fused to the 5′-end of the last gene such that in the RNA precursor produced from this tRNA gene cluster the 3′-end of $tRNA^{Leu}$ (the second gene) is attached to the 5′-end of the precursor to species 1 RNA (the last gene; [1]). Therefore, the possibility had to be considered that the precursor p2Sp1 is unique to the Δ27 deletion strain and its accumulation did not reflect necessarily normal RNA metabolism in a T4-infected cell. These experiments show that p2Sp1 RNA is a normal intermediate in processing of RNA synthesized from the T4 tRNA gene cluster and they help in the formulation of a pathway of RNA processing in T4 infected cells.

2. EXPERIMENTAL PROCEDURES

All chemicals, buffers and solutions used, and phage propagation and titration procedures were as in [4]. The multiplicity of infection in all experiments was 20. A description of the different T4 deletion mutants used is shown in table 1. The preparation of ^{32}P-labeled RNA molecules was done as in [1,6]. RNA oligonucleotide analysis followed the procedures in [4,7–9]. The bacterial strains used, N3433 *rne*$^+$ and N3431 *rne*, are an isogenic pair of strains [10]. (The *rne* strain was used since it accumulates upon infection with T4, a precursor of the last gene in the tRNA gene cluster.)

3. RESULTS

3.1. *Accumulation of p2Sp1 RNA in an* rne *strain infected with different T4 deletion mutants*

To find out if p2Sp1 RNA is unique to the Δ27

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mutant the various T4 strains listed in table 1 were used to infect the *rne* mutant N3431. In all cases when the infection was carried out at 43°C a labeled RNA molecule appeared in the position of p2Sp1 RNA in the gel. (When the Δ33 mutant was used no such molecule appeared since the gene for species 1 RNA is deleted, table 1.) Using a T4 wild-type phage and the Δ27 deletion, an example is shown in fig.1. The regular pattern of small RNAs found after T4 infection can be seen in the second lane (T4 wild type, rne^+ host) and the pattern obtained when the Δ27 mutant infects normal *E. coli* cells is shown in lane 4. One can see the 3 RNAs: species 1, $tRNA^{Gln}$ (the faster migrating band in the tRNA region of the gel) and $tRNA^{Leu}$ (the slower migrating band). Other RNAs also appear. Since these RNAs can be seen also when the deletion Δ33 infects the same host (see table 1 and [11]), they are probably derived from other regions of the T4 chromosome. In the 2nd lane (T4, wild-type) all the RNAs observed in lane 4 (T4Δ27) can be found plus some precursors and species 2 RNA. In the tRNA region the band that appears below $tRNA^{Gln}$ is $tRNA^{Gly}$ [12], and the band that appears in the position of $tRNA^{Gln}$ is very prominent since it contains 5 tRNAs [12]. $tRNA^{Ser}$ that migrates slower than $tRNA^{Leu}$ is not always clearly detected after 40 min of labeling. In lanes 1 and 3 one can clearly observe the accumulation of p2Sp1 RNA at 43°C, the non-permissive temperature for the *rne* strain.

Table 1

Genes in the tRNA region of various T4 deletion mutants

T4 strain	tRNA species									
	Gln	Leu	Gly	Pro	Ser	Thr	Ile	Arg	Sp2	Sp1
T4	+[a]	+	+	+	+	+	+	+	+	+
Δ27	+	+	−	−	−	−	−	−	−	+
Δ8	+	+	−	−	−	+	+	+	+	+
Δ53	+	+	+	−	−	+	+	+	+	+
Δ119	−	−	−	−	−	−	−	+	+	+
Δ33	−	−	−	−	−	−	−	−	−	−

[a] + denotes presence and − denotes absence of the particular gene

The assignments are according to [1–3,5]. The genes are arranged in the order of their appearance in the genome with respect to transcription

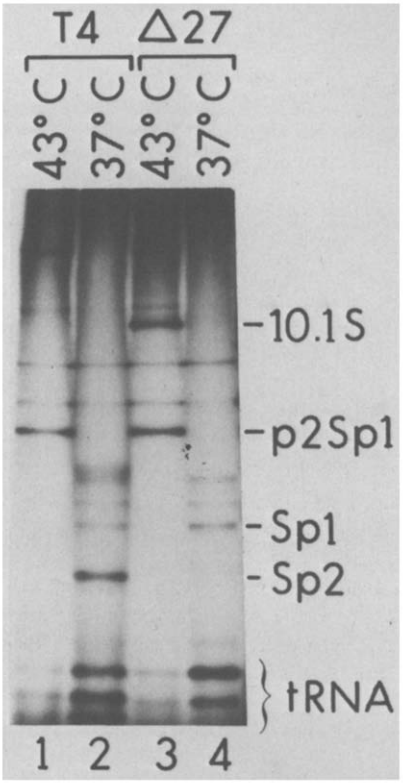

Fig.1. p2Sp1 RNA accumulates in an *rne* strain. Strain N3431 (*rne*) was infected with T4 (wild-type) at 37°C or 43°C and with T4Δ27 at 37°C or 43°C. The cells were labeled with 200 μCi/ml of $^{32}P_i$; 300000 cpm of trichloroacetic acid-precipitable material were loaded in each slot of a 5%/8% polyacrylamide gel containing 0.2% SDS and 7 M urea. At the end of the electrophoresis the 5% portion of the gel was removed, the 8% portion was dried and autoradiographed. The different RNAs accumulating in the cells are indicated. The middle band at the tRNA region comprises a mixture of 5 tRNAs [12] (see text). Labeling was carried out for 40 min. At 43°C the cultures were labeled 30 min after being shifted to 43°C.

These experiments have shown that a molecule comigrating with p2Sp1 RNA accumulates in an *rne* host mutant infected with different T4 deletions mutants or with T4 wild-type. They suggested that the Δ27 deletion is irrelevant to the accumulation of this RNA molecule.

3.2. *Structural analysis of RNA molecules accumulated in the different deletion mutants at the p2Sp1 region of the gel*

The p2Sp1 RNA molecules accumulated after

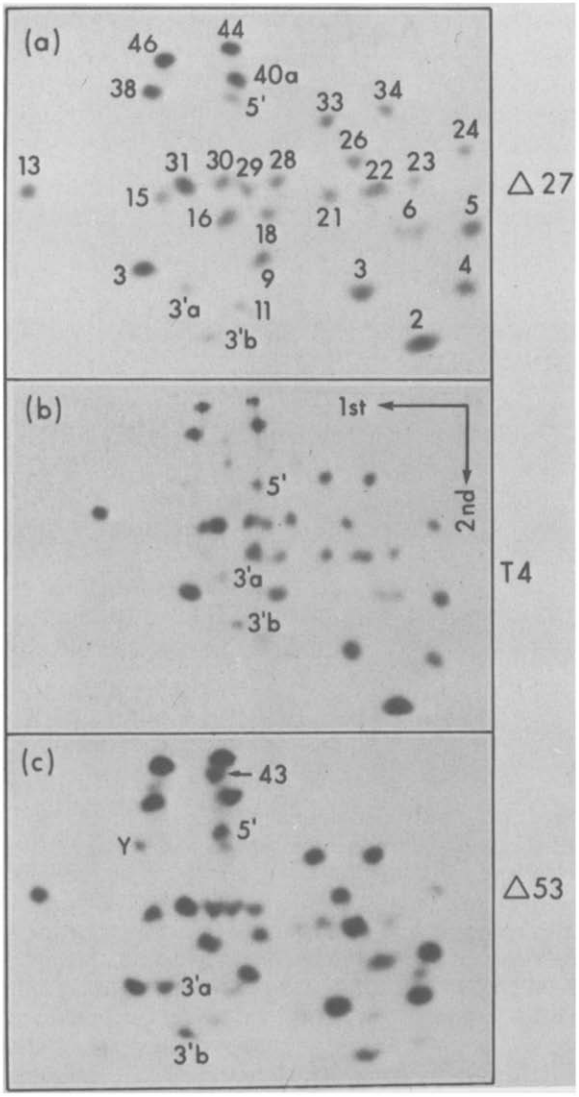

Fig.2. T1 fingerprints of p2Sp1 RNA isolated from *rne* cells infected with different T4 strains. The ^{32}P-labeled RNA molecules were purified from *E. coli* strain N3431 (*rne*) infected with T4Δ27 (a); T4, wild type (b); or T4Δ53 (c). Cells were infected for 30 min at 43°C. The numbers of the oligonucleotides follow those in [1,4]. Further analysis of the T1 generated oligonucleotides was carried out by digestions with pancreatic RNase and nuclease P1. Spots 43 and Y are contaminants described in [1,4].

infection of host strains with various T4 deletion mutants were purified, by using different polyacrylamide concentrations in 4 consecutive preparative gels, digested with ribonuclease T1 and fingerprinted as in [4,6,7]. Fig.2 shows the T1 generated oligonucleotide pattern of p2Sp1 RNA originating from infection of an *rne* mutant with bacteriophages T4Δ27, T4 (wild-type) and T4Δ53. It can be seen that the fingerprints are very similar. Subsequent analysis of the different oligonucleotides by using RNase A [8] has confirmed their identity. The complete analysis of p2Sp1 RNA was presented in [1,4] and since the analysis done here was in complete agreement with the previous analyses, these data are not shown.

Special attention was given to the analysis of the 3′- and 5′-ends of those molecules. The 3′-end of all these RNAs was CUUU(U)U_{OH} as in [4] resembling, as anticipated, a product of termination. All 5′-ends were pCGp which is in agreement with the corresponding DNA sequence [13]. In fig.2 the position of the 5′-end T1 oligonucleotide varies greatly in the different fingerprints (compare fig.2a,b). We found that this is often the case with 5′-end oligonucleotides that contain a 5′-phosphate. The analysis of the 5′-end oligonucleotides from fig.2a–c revealed only pCp and Gp after they were eluted and digested with pancreatic RNase. It is therefore evident that indeed these 3 oligonucleotides are identical.

3.3. *p2Sp1 RNA appears in wild-type* E. coli *cells infected with wild-type T4 phage*

Upon infection of strain N3433 (*rne*$^+$) with T4 phage we observed a low level accumulation of an RNA molecule which migrated to the p2Sp1 RNA region of the gel (fig.3). The accumulation of this RNA was more easily detected when the labeling was carried out at 43°C rather than at 37°C. After 10 min of labeling at 37°C the level of this RNA did not increase any more while at 43°C the relative level of this RNA increased up to about 20 min of labeling. We suspected this molecule to be an RNA precursor that reached its steady state level after apparently 10 min of labeling at 37°C or 20 min at 43°C.

To identify this RNA we labeled a relatively large amount of T4-infected cells (1.5 ml) with 2 mCi/ml of $^{32}P_i$. After 10 min at 37°C and 20 min at 43°C labeling was stopped. The RNA contents of the cells were separated in a 5%/8% polyacrylamide gel containing 0.2% SDS and 7 M urea. In the same gel we ran p2Sp1 RNA as a marker and the appropriate regions of the gel were

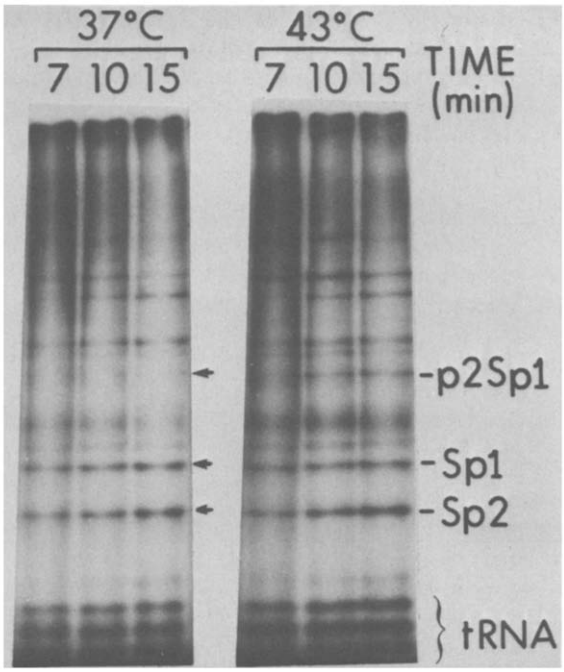

Fig.3. p2Sp1 RNA is an intermediate molecule in wild type cells. Strain N3433 (*rne*$^+$) was infected with T4 (wild-type) and its ^{32}P-labeled RNAs were separated in a 5%/8% polyacrylamide gel containing 0.2% SDS and 7 M urea. Only the 8% portion of the gel was autoradiographed. At both temperatures p2Sp1 RNA can be observed after about 5 min labeling (further details in fig.1). (p2Sp1 at 37°C appears as a very faint band, which is hardly visible in the picture. Its appearance is rather clear in the original autoradiograph.)

excised, the RNA was extensively purified and fingerprinted after digestion with RNase T1 [7]. Both RNA molecules isolated from labeled T4-infected *rne*$^+$ cells at 37°C and 43°C showed the characteristic fingerprint of p2Sp1 RNA.

Since this p2Sp1 RNA molecule could hardly be detected in wild-type cells after longer labeling times and since it had been shown that RNase E activity is reversible when an *rne* culture is shifted back to 37°C [10,14], we infected an *rne* mutant with T4 (wild-type) at 43°C and the culture was labeled with $^{32}P_i$ and 15 min later shifted to 37°C. After either a chase with non-radioactive phosphate, or after blocking RNA synthesis with rifampicin we could find a reduction in label in p2Sp1 RNA concomitant with an increase in the label in species 1 RNA (not shown). This experiment together with the finding of the transient existence of p2Sp1 RNA in wild-type cells suggests that p2Sp1 RNA can mature to species 1 RNA in vivo.

4. DISCUSSION

These results show that the RNA precursor p2Sp1 is a naturally occurring intermediate in the pathway from DNA to mature RNA synthesized from the tRNA gene cluster of T4 bacteriophage.

The molecule was first noticed in the *rne* mutant, when it was infected with the deletion Δ27 [1]. Since this deletion removes 7 of the 10 genes in the tRNA cluster leaving only the first two and the last one intact [1–3,5] and since the deletion apparently joins the last nucleotide in the tRNA^{Leu} gene with the first nucleotide of the precursor of species 1 RNA, that accumulates in an *rnp* mutant [1,2], it was very legitimate to be concerned with the possibility that this precursor could be specific for the Δ27 deletion infecting an *rne* mutant. However, the experiments presented here show that this is not the case.

This precursor (p2Sp1 RNA) seems to be a genuine intermediate in T4 RNA metabolism. Its level is increased at 43°C as compared to 30°C or 37°C (it is higher at 37°C than at 30°C) and is further increased at 43°C in absence of a functional RNase E. It was assumed [1] and shown later [4] that RNase F matures p2Sp1 RNA to Sp1 RNA. This cleavage is apparently less efficient at 43°C as compared to 37°C and is further reduced in the absence of active RNase E.

The reason why the absence of a functional RNase E leads to the accumulation of p2Sp1 RNA even though RNase E does not cleave p2Sp1 RNA [1] can be explained in at least two ways:

(1) By competition of the mutant RNase E with RNase F [1]; or
(2) By assuming the existence of a complex including RNase E and RNase F.

In the complex, the mutated RNase E can affect the activity of RNase F. We prefer the later possibility, which is also supported by a number of other observations including an efficient in vitro cleavage of p2Sp1 RNA by an *rne* cell extract at the non-permissive temperature for RNase E [1], and the inability of extracts of *rne* and *rnc* mutants to

perform RNase P reactions at elevated temperatures [1,15].

Thus, these studies establish p2Sp1 RNA as a normal intermediate in the maturation pathway of RNA in the T4 tRNA gene cluster and further support the notion of an RNA processing complex in the *Escherichia coli* cell.

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