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**Nucleotide sequence and stability of the RNA component of RNase P from a temperature-sensitive mutant of *E. coli***

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**ABSTRACT**

The gene coding for the RNA component of RNase P was cloned from a temperature-sensitive mutant of *Escherichia coli* defective in RNase P activity (ts709) and its parental wild-type strain (4273), and the complete nucleotide sequences of the gene and its flanking regions were determined. The 5'- and 3'-terminal sequences of the RNA component were determined and mapped on the DNA sequence. The mutant gene has GC-to-AT substitutions at positions corresponding to 89 and 365 nucleotides downstream from the 5' terminus of the RNA sequence. Comparing to the wild-type RNA, the mutant RNA is less stable and rapidly degraded *in vivo* and *in vitro*.

**INTRODUCTION**

Ribonuclease P is an endonuclease that cleaves tRNA precursors to generate the 5' termini of mature tRNA molecules (1,2). This enzyme is known to contain an essential RNA component that can be separated from the protein component and subsequently reassembled to reconstitute the enzyme activity (3,4). The essential role of RNase P in *Escherichia coli* has been demonstrated by the isolation of thermosensitive mutants (5,6). At restrictive temperature, the biosynthesis of virtually all tRNA species is abolished and various tRNA precursors accumulate in the mutants (7,8). The mutants we isolated have been mapped at two distinct loci (*rnp*) on the *E. coli* chromosome (9,10). Among the mutants, ts241 that maps at approximately 78 minutes (*rnpA*) and ts709 that maps at 69 minutes (*rnpB*) on the genome have been most extensively characterized (7,8). On the basis of *in vitro* reconstitution experiments, Kole *et al.* (11) have reported that the protein component from ts241 and the RNA component from ts709 may account for the thermosensitive properties of RNase P of these mutants. Recently, Reed *et al.* determined the nucleotide sequence of the gene encoding the RNA component of RNase P (M1 RNA) and the sequence of M1 RNA of 375 nucleotides in length (12).

It remains to be seen, however, whether ts709 represents a mutant of the gene for the RNA component, since attempts to complement the thermosensitive

phenotype of ts709 with a plasmid containing the wild-type gene for 10Sb RNA which is likely to be the same as M1 RNA were unsuccessful (13,14). It has also been shown that the yield of M1 RNA was much reduced in the mutant cells even after the cells were grown at the permissive temperature (12,13,15). The results, however, do not necessarily indicate that ts709 has mutation(s) in the gene for the RNA species, because the production of the RNA molecule could also be affected by mutation(s) in gene(s) different from that for the RNA component. We have determined the complete nucleotide sequence of the *in vitro* transcript of the gene for the RNA component of RNase P and shown that the mature RNA molecule is produced from the transcription product by some processing event (16). Thus, it is possible, for example, that the yield of the mutant RNA is reduced by mutation(s) in the gene(s) for the processing enzyme(s). It is expected that the nucleotide sequence of the mutant RNA may provide crucial information on these problems.

In this paper, we report that the RNA component of RNase P is 377 nucleotides long and the mutant RNA has two base substitutions, indicating that ts709 is a mutant of the gene for the RNA component of RNase P. In addition, we also show that the biosynthesis (transcription and processing) of the mutant RNA is normal as the wild-type RNA but the mutant RNA is much less stable *in vitro* as well as *in vivo*, comparing to the wild-type RNA.

### MATERIALS AND METHODS

*Bacterial and Bacteriophage Strains and Growth Media.* *E. coli* 4273, ts709, ts709arg<sup>-</sup> have been described (6,7,10). *E. coli* BHB2688 and BHB2690 used for *in vitro* packaging of recombinant DNA were obtained from B. Hohn. *E. coli* Q13, LE392, JCI1553/F'JCH5 and other strains were from our laboratory stocks.  $\lambda$ gtWES was supplied from P. Leder. Growth media and low-phosphate medium used for preparation of <sup>32</sup>P-labeled cells were described previously (8,17). Phages were purified as described (17).

*DNA Preparation and Hybridization Procedures.* Phage DNA and *E. coli* DNA were prepared as described (17). RNA-DNA hybridization was performed according to the procedure described by Wahl *et al.* (18). Some minor modifications employed were described previously (17).

*Purification of RNase P.* RNase P was purified from *E. coli* Q13 as will be detailed elsewhere. In short, the enzyme was purified from the 100,000 *g* supernatant fraction of cells using the assay system described previously (19). The purification steps were essentially the same as described by Shimura *et al.* (19) with the following modifications. After the second DEAE-

cellulose chromatography, the enzyme fractions were chromatographed on columns of Sephadex G-200, Sepharose 4B, and n-Octyl-Sepharose. For the isolation of the RNA component from the enzyme, the enzyme preparation was electrophoresed on 10% polyacrylamide gel in 25 mM tris-glycine (pH 7.0). The running buffer was 25 mM tris-glycine (pH 9.0). RNase P was identified after briefly soaking in a solution of 1  $\mu\text{g/ml}$  ethidium bromide under ultraviolet irradiation. The enzyme was recovered from the gel and extracted with phenol. The RNA was recovered by ethanol precipitation.

For the preparation of uniformly labeled RNA component, cells (4273) were labeled with  $^{32}\text{P}$ -orthophosphate (Japan Isotope Corporation) at 37°C for 4 hr as described previously (8). The 100,000 g supernatant of the extract of the labeled cells was mixed with the supernatant from the unlabeled cells and RNase P was purified as described above.

*Cloning of the Gene for the RNA Component of RNase P.* *E. coli* DNA was digested with *EcoRI* and the digests were extracted with phenol. The *EcoRI* fragments were ligated to the terminal *EcoRI* fragments of  $\lambda\text{gt}$  DNA. The resulting DNA was packaged into phage particles according to the procedure of B. Hohn (20). The phage was plated on *E. coli* LE392 and the recombinant clones containing the gene for the RNA component were identified by the *in situ* screening procedure described previously (21). The RNA extracted from the purified RNase P preparation was labeled, after alkaline phosphatase treatment, with  $[\gamma\text{-}^{32}\text{P}]\text{ATP}$  (Amersham) at its 5' terminus by polynucleotide kinase, purified by electrophoresis on 8% polyacrylamide gel, and used as a hybridization probe.

*Nucleotide Sequencing Procedures.* DNA sequence analysis of restriction fragments was performed according to the method of Maxam and Gilbert (22).

For the analysis of the 5'-terminal sequence of the RNA component of RNase P, the 5' end of the RNA from the enzyme was labeled as described above. The labeled RNA was purified by electrophoresis on 8% polyacrylamide gel containing 8M urea. Partial nucleotide sequence of the 5'-terminal region of the RNA was determined according to the method described by Donis-Keller and colleagues (23,24).

For the analysis of the 3'-terminal sequences of the RNA component, the RNA was labeled at its 3' end with  $[\text{5}'\text{-}^{32}\text{P}]\text{pCp}$  (Amersham) by T4 RNA ligase as described (25). The labeled RNA was purified by gel electrophoresis as above, and subsequently digested with RNase T1. The digests were fractionated by electrophoresis on 20% polyacrylamide gel containing 8M urea. After autoradiography, radioactive bands were recovered from the gel. The end-

labeled oligonucleotides were subjected to partial alkaline hydrolysis according to the protocol described by Donis-Keller *et al.* (23). The digests were fractionated by two-dimensional homochromatography as described (25,26). Chromatography for the second dimension was carried out in the Homo-mix V of Jay *et al.* (26).

Two-dimensional fingerprint, after RNase T1 digestion of  $^{32}\text{P}$ -labeled RNA, was made according to the standard procedure (27,28). Oligonucleotides were analyzed as described (8).

*In Vitro Transcription and Processing.* The procedures for *in vitro* transcription of the gene for the RNA component and for *in vitro* processing of the transcripts were described previously (16).

### RESULTS

*Cloning of the Gene for the RNA Component of RNase P from E. coli 4273 and ts709.* The RNA component was extracted from a highly purified RNase P preparation. The RNA was purified by electrophoresis on 8% polyacrylamide gel containing 8M urea and the RNA band of approximately 370 nucleotides long was recovered from the gel. When this RNA was incubated with the protein component of RNase P, RNase P activity was reconstituted (data not shown). Therefore, this RNA was concluded to represent the RNA component of the enzyme. The RNA preparation was labeled at its 5' terminus with [ $\gamma$ - $^{32}\text{P}$ ]-ATP by polynucleotide kinase and purified again by electrophoresis on 8% polyacrylamide gel. In the autoradiogram of the gel, the presence of a single major band was noted (data not shown). The RNA band was recovered from the gel and used as a hybridization probe to identify the gene for the RNA component of RNase P.

When DNAs from *E. coli* 4273 and ts709 were individually digested with *EcoRI*, electrophoresed on a 1% agarose gel, and subsequently hybridized with the probe by Southern blotting, a single hybridizable band of approximately 10 kilobase pairs (kb) in length was detected (Fig. 1a). The 10-kb *EcoRI* fragments were cloned from the two strains; the clone containing the wild-type DNA fragment was designated  $\lambda\text{grnpR-W}$  and the clone containing the mutant DNA fragment was designated  $\lambda\text{grnpR-709}$  (Fig. 1b).

*Nucleotide Sequence of the Gene for the RNA Component from the Wild-Type Strain.* The localization of the RNA gene in the insert of  $\lambda\text{grnpR-W}$  was pursued by Southern blot hybridization using the end-labeled RNA as a probe. When the cloned fragment was digested with *HincII*, the RNA was specifically hybridized with a fragment of 2.8 kb in length (data not shown).

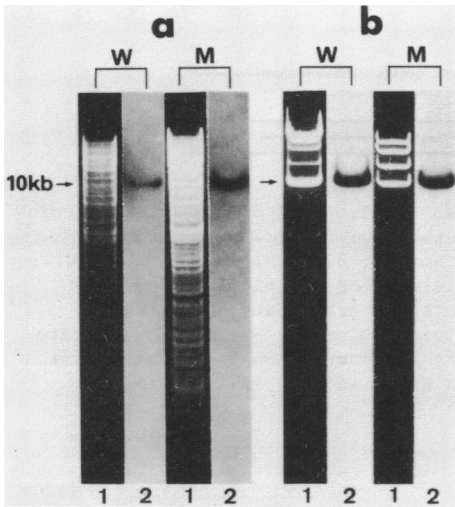


Figure 1. Identification of the gene encoding the RNA component of RNase P. a. DNAs (3  $\mu$ g) from *E. coli* strains 4273 (lane W) and ts709 (lane M) were individually digested with *EcoRI*, and subsequently electrophoresed on a 1% agarose gel. The DNA bands were visualized by staining with ethidium bromide (lane 1). The DNA fragments were transferred onto nitrocellulose filter and hybridized with the end-labeled RNA from purified RNase P ( $9 \times 10^4$  cpm input, about  $4 \times 10^5$  cpm/ $\mu$ g specific activity). After hybridization, the filter was autoradiographed (lane 2). b. DNAs (0.5  $\mu$ g) from  $\lambda$ grnpR-W (lane W) and  $\lambda$ grnpR-709 (lane M) were individually digested with *EcoRI* and the digests were subjected to Southern blot hybridization as in a. Lanes: 1, *EcoRI* digests of DNA; 2, autoradiogram of the Southern blot of lane 1.

This fragment (designated *Hinc2.8kb*) was mapped with various restriction endonucleases such as *BstNI*, *Sau3AI*, *PstI*, *SmaI*, and *SstII*. The results of the mapping experiments are summarized in Fig. 2. The region corresponding to the RNA sequence was also mapped by Southern blot hybridization of these restriction fragments. The gene for the RNA component was found to be included within a region of approximately 600 base pairs (bp) between the *SstII* site at 1.4 kb and the *BstNI* at 2.0 kb in the figure.

From the mapping studies with the region spanning the *SstII* and *BstNI* sites, it was possible to generate fragments and strands that would overlap all restriction sites within this region. The end-labeled fragments employed in the sequence experiments and DNA sequencing strategy in this region on the map are summarized in Fig. 2. The nucleotide sequence of this region thus determined is shown in Fig. 3. This sequence is consistent with that of the corresponding region reported by Reed *et al.* (12) with an exception of two nucleotides. In the sequence determined in the present studies, there is an additional AG sequence 31 bp downstream from the *Sau3AI* site marked by an arrow in the figure. As will be described later, this is also the case with the sequence of the corresponding region from ts709.

To determine the nucleotide sequence of the RNA component, the terminal

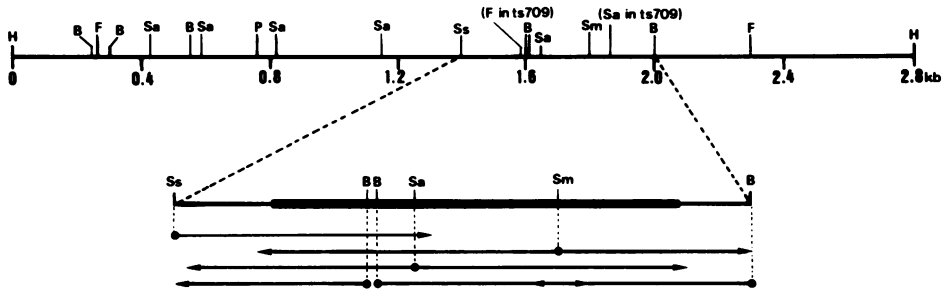


Figure 2. Restriction map of the *Hinc*2.8kb fragment and diagram of protocols for sequencing end-labeled restriction fragments. The following restriction endonuclease cleavage sites are shown: H, *Hinc*II; B, *Bst*NI; F, *Fok*I; P, *Pst*I; Sa, *Sau*3AI; Sm, *Sma*I; Ss, *Sst*II. The 5' termini of restriction fragments labeled with  $^{32}$ P are indicated by small dots and arrows attached to them show the directions of sequencing. The lines behind the arrowheads indicate portions that actually yielded satisfactory sequences. The restriction sites in parentheses indicate the cleavage sites detected only in ts709 DNA.

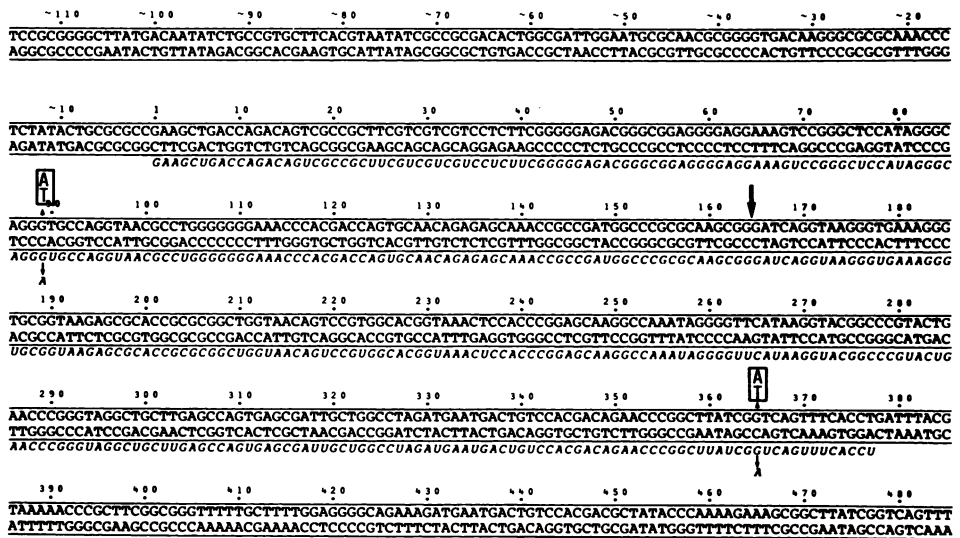


Figure 3. Nucleotide sequences of the gene for the RNA component of RNase P and its flanking regions. The nucleotide sequences of the RNA component is presented in italics. The 5' end of the RNA is marked +1. The cleavage site for *Sau*3AI is indicated by an arrow. The base substitutions in the ts709 gene and the mutant RNA molecule at positions 89 and 365 are indicated in the figure.

sequences of the RNA molecule were partially determined and mapped on the DNA sequence. The 5' terminus of the RNA molecule prepared from purified RNase P was labeled with [ $\gamma$ -<sup>32</sup>P]ATP and the RNA was purified by polyacrylamide gel electrophoresis as described above. The end-labeled RNA was subjected to partial digestion with various RNases and the digests were electrophoresed on 20% polyacrylamide gel containing 8M urea as described previously (16). The sequence of the first 16 nucleotides of the RNA molecule was found to be (5')GAAGCUGACCAGACAG- (data not shown). Comparison of this sequence with the DNA sequence determined above allowed us to identify the position in the DNA sequence that corresponded to the 5'-terminal G residue of the RNA molecule. The G residue is 164 bp upstream from the *Sau*3AI site as shown in Fig. 3. It is worth noting that M1 RNA has pppG at its 5' terminus, according to Reed *et al.* (12).

The 3'-terminal sequence of the RNA molecule was partially determined and also mapped on the DNA sequence. The RNA molecule was end-labeled with [5'-<sup>32</sup>P]pCp by T4 RNA ligase and purified as described above. The labeled RNA was digested to completion with RNase T1 and the digests were electrophoresed on 20% polyacrylamide gel containing 8M urea. Partial alkaline hydrolysates of the end-labeled RNA were electrophoresed in parallel as size marker. Two radioactive bands were detected in the gel; one was 9 nucleotides in length and the other was 8 nucleotides long (data not shown). The yields of the smaller oligonucleotide were variable in different experiments and usually less than those of the larger oligonucleotide. These oligonucleotides were individually analyzed by two-dimensional homochromatography after partial alkaline hydrolysis and by paper electrophoresis after complete digestion with RNase T2 as described previously (16,25). The results show that the larger and smaller oligonucleotides are 5'-UUUACCUC and 5'-UUUACCCC, respectively, where the 5'-terminal Us are linked to G and the 3'-terminal Cs are originated from pCp ligated to the 3' end of the RNA molecule (data not shown). Thus, the larger oligonucleotide must represent an RNA sequence of 5'-UUUACC, which is consistent with the result of Reed *et al.* (12) who determined the sequence of the 3'-terminal RNase T1 oligonucleotide of uniformly labeled M1 RNA by fingerprint analysis. A DNA sequence corresponding to the RNA sequence (5'-GUUUCACCU) is present in the DNA sequence 369-377 bp downstream from the position corresponding to the 5' terminus of the RNA sequence. It is likely that the smaller oligonucleotide was generated by removal of the 3'-terminal U residue of the RNA molecule during the purification of the enzyme. The presence of heterogeneous ends in M1 RNA

were also reported by the previous workers.

On the basis of these sequence analyses, the entire sequence of the RNA component of RNase P was determined as shown in Fig. 3. The RNA is 377 nucleotides in length. This is 2 nucleotides longer than the length of M1 RNA previously reported (12), due to the additional AG sequence in the middle of the RNA coding region as described above.

*Nucleotide Sequence of the Gene for the RNA Component from ts709.* To determine the nucleotide sequence of the RNA component of RNase P from ts709, the insert of  $\lambda$ grnpR-709 was similarly analyzed as that of the wild-type clone. Southern blot hybridization revealed that the region coding for the RNA sequence was also included within the region of about 600 bp between the *Sst*II and *Bst*NI sites in the *Hinc*2.8kb fragment of the mutant DNA. Thus the nucleotide sequence of this region was determined using the same protocols employed for the sequence analysis of the wild-type gene (Fig. 2). We found two base-pair substitutions in the mutant gene; the G-C pairs located 89 and 365 bp downstream from the 5' terminus of the RNA coding sequence in the wild-type gene were substituted by A-T pairs as illustrated in Fig. 3.

We have additional evidence that supports the results of the sequencing experiments. As seen in Fig. 3, the GC-to-AT substitution at position 89 may create a new restriction site for *Fok*I (5'GGATG) in the mutant gene. Similarly, the base substitution at position 365 may create a restriction site for *Sau*3AI (5'GATC) in the mutant gene. Accordingly, the wild-type and mutant genes should show different Southern-hybridization patterns when digested with these restriction enzymes and subsequently hybridized with the <sup>32</sup>P-labeled RNA molecule. When the *Hinc*2.8kb fragment from the wild-type DNA was digested with *Fok*I, a positive band of 2.0 kb in length was observed, while two positive bands of 1.3 kb and 0.7 kb in length were detected in the *Fok*I digests of the *Hinc*2.8kb fragment from ts709 DNA (Fig. 4a). On the other hand, upon digestion with *Sau*3AI, the wild-type DNA showed two positive bands of 1.1 kb and 0.5 kb in length, while the mutant DNA generated three hybridizable bands of 0.9 kb, 0.5 kb, and 0.2 kb in length as shown in Fig. 4b. These results were expected from the restriction map shown in Fig. 2 and the nucleotide sequence shown in Fig. 3. Thus we conclude that the RNA component of RNase P from ts709 has two G-to-A substitutions at positions 89 and 365 from the 5' end of the molecule. Conversely, the results show that *rnpB* is the gene that codes for the RNA component of RNase P.

*Effect of the Base Substitutions on the Amount of the RNA Component in ts709.* It has been shown that the amount of the RNA component of RNase P



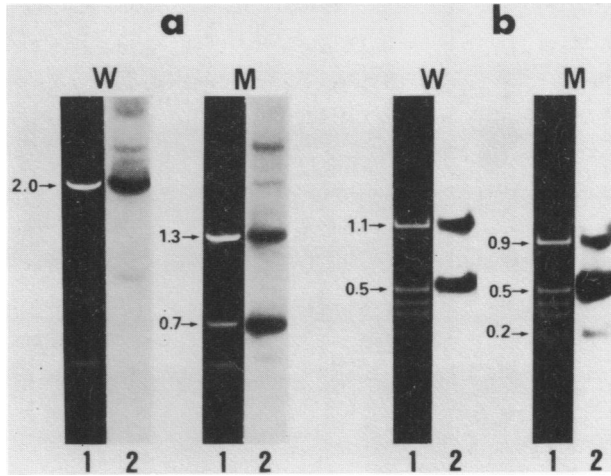


Figure 4. Southern blot analysis of the region encoding the RNA component of RNase P. The *Hinc*2.8kb fragments (0.5  $\mu$ g) from  $\lambda$ grnpR-W (lane W) and from  $\lambda$ grnpR-709 (lane M) were individually digested with *Fok*I (a) or *Sau*3AI (b). The digests were electrophoresed on a 2% agarose gel. Migration is from top to bottom. The DNA fragments were visualized by staining with ethidium bromide (lane 1). The DNA fragments were subjected to Southern blot hybridization as in Figure 1. After hybridization, the filter was autoradiographed (lane 2). The sizes (kb) of the fragments are indicated in the figure.

is considerably reduced in ts709 (12,13,15). The wild-type cells grown at 37°C were labeled with  $^{32}$ P-orthophosphate and RNA was prepared by phenol extraction. When the labeled RNA molecules of approximate molecular sizes of 300-450 nucleotides were fractionated by two-dimensional polyacrylamide gel electrophoresis, several radioactive spots were detected as shown in Fig. 5a. The same RNA spots were observed when cells were grown at 30°C or 42°C. When the spots were individually digested with RNase T1 and analyzed by fingerprinting, one of the spots (marked by an arrow in the figure) showed a fingerprint pattern (Fig. 5d) which was consistent with that of the uniformly  $^{32}$ P-labeled RNA component isolated from RNase P (data not shown). When  $^{32}$ P-labeled RNA from ts709 grown at 30°C was similarly analyzed, little or no material was detected comigrating with the spot corresponding to the RNA component in the two-dimensional gel (Fig. 5b). However, when F'JCH5, an episome carrying the 62-to-70-minute region of the *E. coli* chromosome, was introduced into ts709, the thermosensitive phenotype of the mutant was complemented as reported previously (10) and the RNA spot became clearly detectable as shown in Fig. 5c and 5e. These results are consistent with

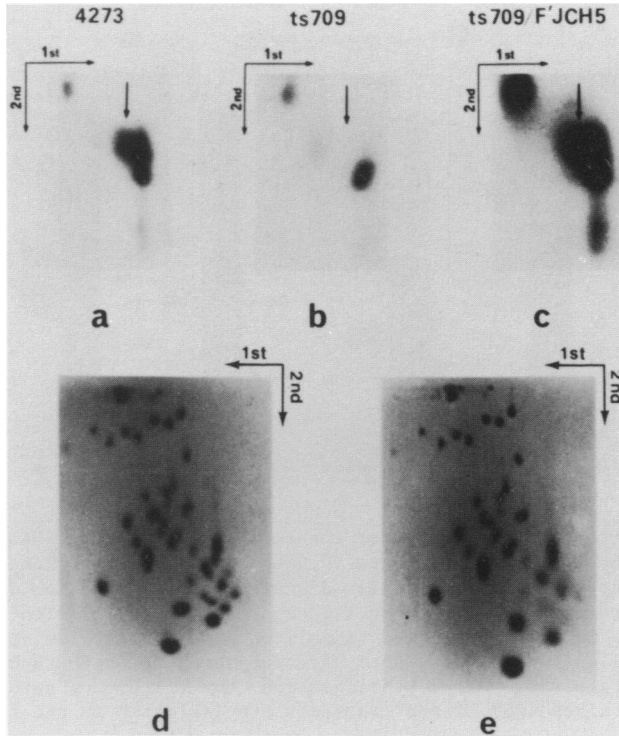


Figure 5. Identification of the RNA species corresponding to the RNA component of RNase P in cells. *E. coli* strains 4273 and *ts709argG<sup>-</sup>/F'JCH5* grown at 37°C and strain *ts709* grown at 30°C were labeled with <sup>32</sup>P-orthophosphate and RNAs were extracted with phenol as described (8). The RNAs were electrophoresed on a 10% polyacrylamide gel. The slices containing RNAs of approximate molecular sizes of 300-450 nucleotides were placed on the top of 12% polyacrylamide gels and electrophoresed as described (8). The gels were autoradiographed (a, b, and c). The RNA spots indicated by arrows in a and c were recovered from the gels and analyzed by fingerprinting after digestions with RNase T1. The RNase T1 fingerprints of the RNA from 4273 and *ts709argG<sup>-</sup>/F'JCH5* are shown in d and e, respectively.

those reported previously (12,13,15).

The two point mutations appear to be responsible for the reduced yields of the RNA component in *ts709*. It is possible that the mutations affect the stability of the RNA in *ts709*. It is also possible that the mutations lower the production of the RNA in *ts709*, since one of the mutational sites is located only 13 nucleotides upstream from the processing site of the transcript of the gene (16). To examine these possibilities, the following experiments were performed. The *Hinc*2.8kb fragments from  $\lambda$ grnpR-W and  $\lambda$ grnpR-

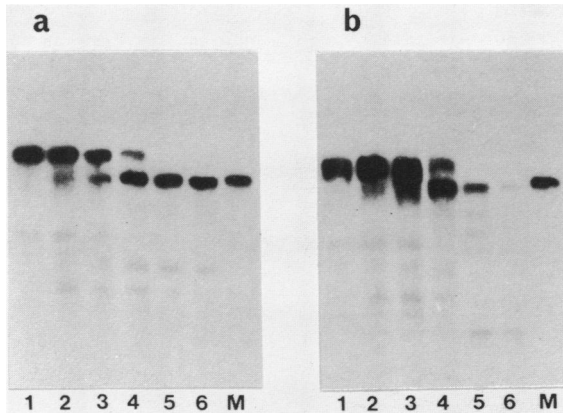


Figure 6. *In vitro* processing of the transcripts of the gene for the RNA component of RNase P. The *Hinc*2.8kb fragments from  $\lambda$ grnpR-W (wild-type) and  $\lambda$ grnpR-709 (ts709) were individually transcribed *in vitro* in the presence of [ $\alpha$ - $^{32}$ P]GTP as the radioactive substrate, and the major transcripts were purified by gel electrophoresis as described (16). The wild-type and mutant transcripts (approximately 2 fmol,  $1 \times 10^4$  cpm for each) were individually incubated at 37°C with the S30 extract under the standard conditions described previously (16). Incubation times (min) were as follows: 0 (lane 1), 10 (lane 2), 30 (lane 3), 60 (lane 4), 120 (lane 5), and 180 (lane 6). After incubation, RNA was extracted with phenol, electrophoresed on 8% polyacrylamide gel/8M urea, and autoradiographed. Migration is from top to bottom. The RNA component isolated from purified RNase P and end-labeled at its 5' terminus with [ $\gamma$ - $^{32}$ P]ATP by T4 polynucleotide kinase was electrophoresed on the same gel (lane M).

709 were individually transcribed *in vitro* and the transcripts of the gene for the RNA component of 413 and 414 nucleotides long were purified as described previously (16). It is worth noting that the template activity of the mutant gene was essentially the same as that of the wild-type gene (data not shown). The transcripts were then individually incubated with an S30 extract from *E. coli* Q13 at 37°C. As shown in Fig. 6a and 6b, the majority of the transcripts were processed to the mature size after 60 min. The mature-size product has the same terminal sequences as the RNA component from RNase P as described (16). The product from the wild-type transcripts was stable for further incubation and much of the products still remained after 3 hr incubation (Fig. 6a). The mutant transcripts were processed similarly as the wild-type transcripts but little product was detected after 3 hr (Fig. 6b). When the transcripts of the wild-type and mutant genes were individually processed *in vitro* and the products recovered from the gel were incubated with the Q13 extract at 30°C or 44°C, we found that the mutant product was

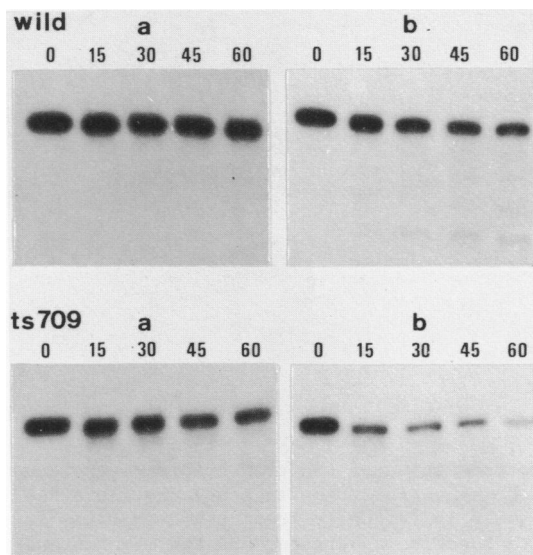


Figure 7. Stability of the *in vitro* synthesized RNA component of RNase P in cell extract. The *Hinc*2.8kb fragments from  $\lambda$ grnpR-W (wild) and  $\lambda$ grnpR-709 (ts709) were individually transcribed *in vitro* and the major transcripts were purified as in Figure 6. The wild-type and mutant transcripts (approximately 0.1 pmol,  $5 \times 10^5$  cpm for each) were incubated at 37°C with the S30 extract for 60 min under the standard conditions (16). After incubation, RNAs were extracted with phenol and electrophoresed on 6% polyacrylamide gel/8M urea. The *in vitro* processed RNAs were recovered from the gel. A portion of each RNA (approximately 1 fmol,  $5 \times 10^3$  cpm) was incubated with the S30 extract, in the reaction mixture employed in the processing reaction (16), at 30°C (panels a) or 44°C (panels b). Incubation times (min) are indicated in the figure. After incubation, RNA was extracted with phenol and electrophoresed on 8% polyacrylamide gel/8M urea. Migration is from top to bottom.

degraded more rapidly at 44°C than at 30°C, comparing to the wild-type product (Fig. 7). These results indicate that the mutant RNA is more labile than the wild-type RNA, and that the instability of the mutant RNA is more profound at the high temperature. Therefore, we conclude that the two base substitutions may lower the stability of the RNA component but do not affect much the production of the RNA.

#### DISCUSSION

We have cloned the gene coding for the RNA component of RNase P from *E. coli* strains 4273 and ts709, a thermosensitive *rnpB* mutant. The gene was identified by blot hybridization using the RNA molecule isolated from a

highly purified RNase P preparation. This RNA is active in the reconstitution of RNase P activity when incubated with the protein component, indicating that it represents the RNA component of the enzyme. From the blot hybridization experiment with *E. coli* DNA, the gene for the RNA species is present only in an *EcoRI* fragment of 10 kb long. It is likely that the RNA is coded for by a single gene in the *E. coli* genome.

The sequence of the wild-type gene and its flanking regions determined in the present work is consistent with that reported by Reed *et al.* (12), except that there is an additional AG sequence in the middle of RNA coding region in the gene sequence we determined. The same extra sequence is also present in the corresponding region from ts709. Thus, the RNA component has been shown to be 377 nucleotides long in our case, while the previous workers have reported that M1 RNA is 375 nucleotides long. It remains to be elucidated why the two different sequences were obtained by the two groups. It is possible that the difference is due to the strains employed as the source of the gene by the two groups. If this is the case, the two extra nucleotides might not be important for the function of the RNA component.

The nucleotide sequence of the gene from ts709 differs from that of the wild-type strain by two base pairs. In the mutant gene, the G-C pairs located 89 and 365 bp downstream from the position corresponding to the 5' terminus of the RNA sequence in the wild-type gene are substituted by A-T pairs. Thus, the RNA component from the mutant has two G-to-A transitions at the two positions. According to the model for the secondary structure of M1 RNA proposed by Reed *et al.* (12), the two mutational sites in the ts709 RNA are in base-paired regions. It is not known, however, whether the two point mutations in the ts709 gene are directly responsible for the thermosensitive nature of the mutant enzyme. Attempts to rescue the thermosensitive growth of ts709 with a plasmid containing the wild-type gene for 10Sb RNA which is likely to be the same as M1 RNA were unsuccessful (13,14). It is possible, therefore, that ts709 may have multiple mutations not only in the gene for the RNA component but also in some other gene(s), and that the mutation(s) in the latter gene(s) are more directly responsible for the thermosensitive growth of the mutant. This possibility is likely, because the wild-type cells were heavily mutagenized for the isolation of the mutant (6). In fact, among the revertants of ts709 that grow at 42°C, we have found at least a few strains whose RNase P activity is still defective as ts709. The analyses of such revertants will be described elsewhere.

It has been shown that the yield of the RNA molecule is considerably

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lower in ts709 even at the permissive temperature. Reed et al. have shown that M1 RNA is detectable in ts709 at 30°C by Northern blot hybridization but its level is much reduced at 44°C (12). We have found that the mutant gene is as active as the wild-type gene when transcribed *in vitro* and both genes give the transcription products of identical chain lengths, and that the transcripts of both genes are processed *in vitro* to the mature size equally well. However, after processing reaction, the mutant product is rapidly degraded in the crude extract and the degradation of the mutant RNA is more rapid at 44°C than at 30°C. Thus, comparing to the wild-type RNA, the mutant RNA appears to be more sensitive to temperature shift and more susceptible to nuclease attack in the cell extract at the higher temperature. It is highly likely that the base substitutions are responsible for the instability of the mutant RNA. This may explain why the yield of the RNA component of RNase P is far reduced in ts709 *in vivo*. It remains to be clarified, however, whether both of the two substitutions are responsible for the instability of the RNA molecule. This may be answered if we isolate revertants at each of the mutational sites. The experiments along this line are in progress in our laboratory and will be published elsewhere.

We have at least several other temperature-sensitive mutants which have been isolated independently and mapped at the *rnpB* locus on the chromosome. In some of the mutants (eg. ts2418), the yield of the RNA component of RNase P is as normal as in the wild-type cells. It is of interest to examine the mutational alterations in the gene for the RNA component and their effects on the enzyme function. The structural analysis of the RNA component from the wild-type and thermosensitive *rnpB* mutant strains may give some insight into the role of the RNA component on substrate recognition of RNase P.

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