STUDIES ON T4-tRNA BIOSYNTHESIS: ACCUMULATION OF PRECURSOR tRNA MOLECULES IN A TEMPERATURE SENSITIVE MUTANT OF *ESCHERICHIA COLI*

H. SAKANO, Y. SHIMURA and H. OZEKI
Department of Biophysics, Faculty of Science, Kyoto University, Kyoto, Japan

Received 14 January 1974

1. Introduction

Bacteriophage T4 is known to direct the synthesis of eight distinct tRNA species [1,2]. The structural genes for these tRNAs seem to be clustered in a small region of the T4 genome, although it is not as yet clear whether these genes are transcribed as a single unit [3,4]. In the course of characterization of temperature sensitive mutants of *E. coli* defective in tRNA biosynthesis, we have demonstrated that none of the mature T4 tRNAs is synthesized in one of the mutants [5]. In the present paper, we report that in this mutant (TS241) a dimeric precursor molecule containing serine and proline tRNAs and two monomeric precursor molecules for glycine tRNA and an unidentified ϵ tRNA of T4 phage are accumulated at restrictive temperature. Moreover, all of these precursor molecules were cleaved in vitro (at both low and high temperatures) by crude extracts from wild type *E. coli* into their corresponding tRNA molecules. If, however, the crude extracts prepared from the mutant were employed, the cleavage reactions did not occur at high temperature, although they did occur at low temperature. Thus, the same enzyme is involved in cleaving the dimeric and the monomeric precursor molecules.

2. Materials and methods

E. coli 4273 (su^-, lac^-_{am}, $T6^r_{am}$, $BF23^r_{am}$) and TS241, a temperature sensitive mutant of 4273, defective in tRNA biosynthesis were described elsewhere [5]. Low phosphate medium used for ^{32}P labelling, phage infection, extraction of ^{32}P-labelled RNA, and electrophoresis on polyacrylamide slab gel were also described earlier [5]. A dialyzed S30 preparation of *E. coli*, used for the in vitro cleaving reactions of precursor tRNA molecules, was prepared as described by Nathans [6]. Fingerprint analysis of T1-ribonuclease digests of RNA was performed according to the method described by Barrell [7].

3. Results and discussion

On infection of *E. coli*, phage T4 induces the synthesis of eight unique tRNA species. Some of the T4 tRNA species can be easily purified by electrophoresis on a 10% polyacrylamide gel as shown in fig. 1d. Previous studies [2,8,9] have shown that band 3 is a serine tRNA; band 4 is a leucine tRNA; band 6 is a glycine tRNA; and band 5 is a mixture of five species of tRNA that can be resolved by 20% polyacrylamide gel electrophoresis. When TS241 was infected with T4 and subsequently grown at high temperature, the synthesis of mature T4 tRNA molecules was drastically changed as judged by the electrophoretic pattern on a 10% polyacrylamide gel (fig. 1a). There was a sharp increase of band A and the appearance of two possibly new bands (C and D) was apparent. Also noted in fig. 1a were some increase of band B and additional new bands between band A and the origin. This abnormal gel pattern was not seen if the phage encoded RNA was made in the same mutant at 30°C as was reported previously [5].

When the T4 RNA preparation from the mutant grown at 42°C was incubated with an S30 cell-free extract from *E. coli* Q13 ($RNase\ I^-$) and the reaction

North-Holland Publishing Company – Amsterdam

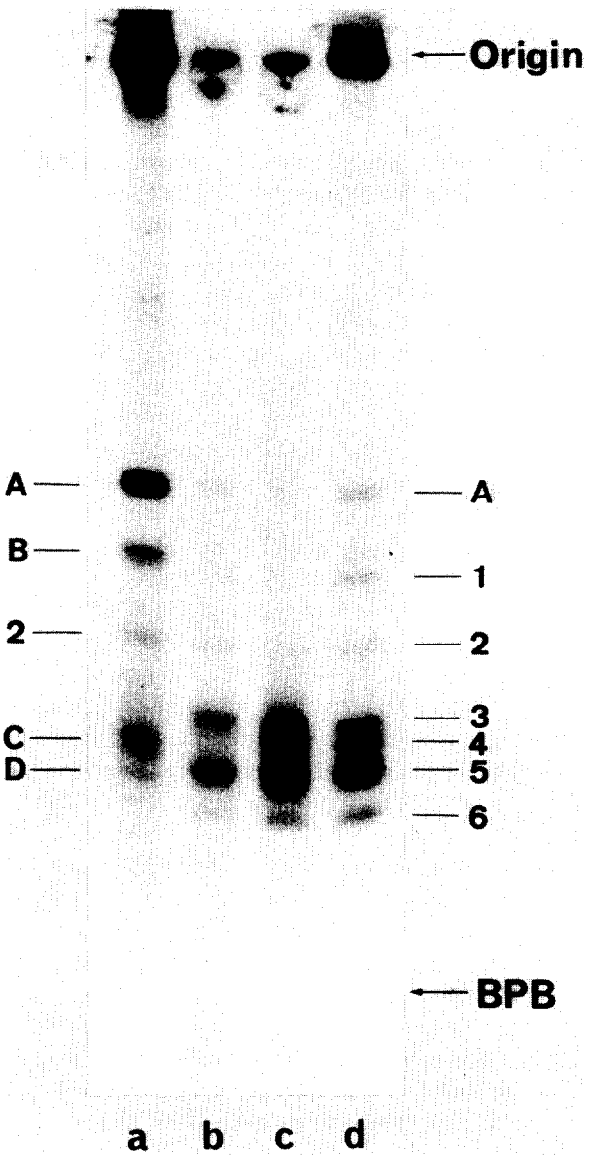

Fig. 1. Autoradiogram of 10% polyacrylamide gel electropherogram. ^{32}P-labelled RNA was extracted from T4 infected cells and electrophoresed as described earlier [5]. a: T4 RNA made in TS241 at 42°C. d: T4 RNA made in 4273 at 42°C. b: The ^{32}P-labelled preparation from a (T4-infected TS241) was incubated with the S30 cell-free extract from *E. coli* Q13 at 37°C for 30 min. The reaction mixture contained, in 0.3 ml, 10 mM Tris–Cl (pH 7.8), 0.1 mM EDTA, 5 mM $MgCl_2$, 0.1 mM 2-mercaptoethanol, and 30 μl S30. After incubation, RNA was extracted with phenol, washed several times by ethanol precipitation. c: RNA from d (T4-infected 4273) was treated with the Q13 extract as in b.

products were analyzed on a 10% polyacrylamide gel, bands A and B were greatly reduced and at least three additional bands appeared in the tRNA region (fig. 1b). Among the three bands, the slowest migrating species and the fastest migrating species were found to correspond to serine and glycine tRNAs, respectively, as judged by electrophoretic mobilities on the gel as well as by two-dimensional fingerprint patterns of T1 RNase digests (data not shown). The band in the middle was further resolved by 20% polyacrylamide gel electrophoresis; two components were found, one with the mobility of proline tRNA as a major component and a minor component with the mobility of ϵ tRNA, a tRNA of unknown specificity [2]. Although sequence analysis of these two components is currently in progress, it is most likely that the major one corresponds to proline tRNA and the minor one to ϵ tRNA. So far no other T4 tRNA species have been detected after treatment of the T4 RNA preparation from TS241 grown at 42°C with the *E. coli* extract.

In order to identify specific RNA molecules from which these four tRNA species were derived, we extracted band A from the gel shown in fig. 1a, treated with the Q13 extract, and fractionated by polyacrylamide gel electrophoresis. As shown in fig. 2,

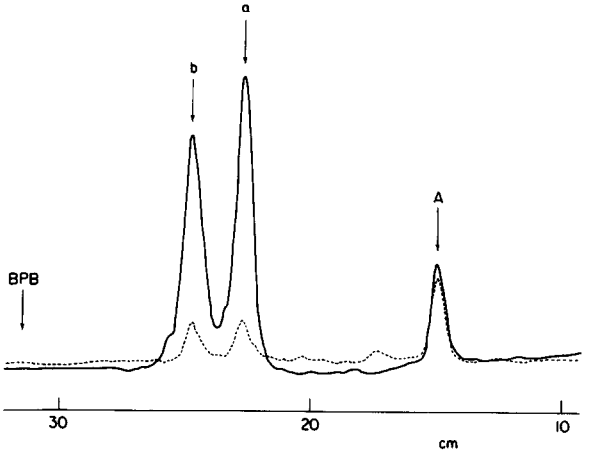

Fig. 2. Densitometer tracings of the autoradiograms of ^{32}P-labelled T4 RNA. Separation of the cleavage products of band A from T4 infected TS241 (solid line) and from T4 infected 4273 (dashed line) was performed on a 10% polyacrylamide gel. Band A was extracted from the gel of fig. 1(a) and (d) and treated with the Q13 extract as in fig. 1. The radioautograms were traced with a Joyce–Loebl microdensitometer.

the majority of band A was cleaved, upon incubation in the extract, into two RNA fragments, one of which (band a) has the same mobility as serine tRNA and the other corresponds to proline tRNA. The cleavage of band A was not seen without the *E. coli* extract. Thus, band A is probably the dimeric precursor molecule for serine and proline tRNAs described by Guthrie et al. [9]. It is worth noting that a portion of band A was uncleaved even after prolonged incubation with the extract. Apparently this residual band is different from the dimeric RNA molecule. Band A from wild type cells (fig. 2, dashed line) also yielded the same two RNA species after incubation, although the amounts of reaction products relative to the residual band A were much reduced. This indicates that the dimeric molecule had accumulated in the mutant at high temperature. When band D (fig. 1a) was extracted from the gel and incubated with the Q13 extract, it was converted to a band, the mobility of which is the same as band 6 (glycine tRNA) on a 10% polyacrylamide gel (fig. 3.1). Thus, glycine tRNA seems to be derived from band D. Since the electrophoretic mobility of band D suggests that this RNA is several nucleotides longer than mature T4 glycine tRNA, we tentatively conclude that band D is a monomeric precursor for glycine tRNA. Band C is broader than any other bands and was found to contain several RNA species, though in small quantities, as judged by two-dimensional polyacrylamide gel electrophoresis (data not shown). Upon incubation with the Q13 extract, band C was converted to a band, the mobility of which was the same as ϵ tRNA on a 20% polyacrylamide gel. Thus, band C appears to contain a monomeric precursor for ϵ tRNA. Sequence analysis of these precursor molecules is currently in progress and will be published elsewhere.

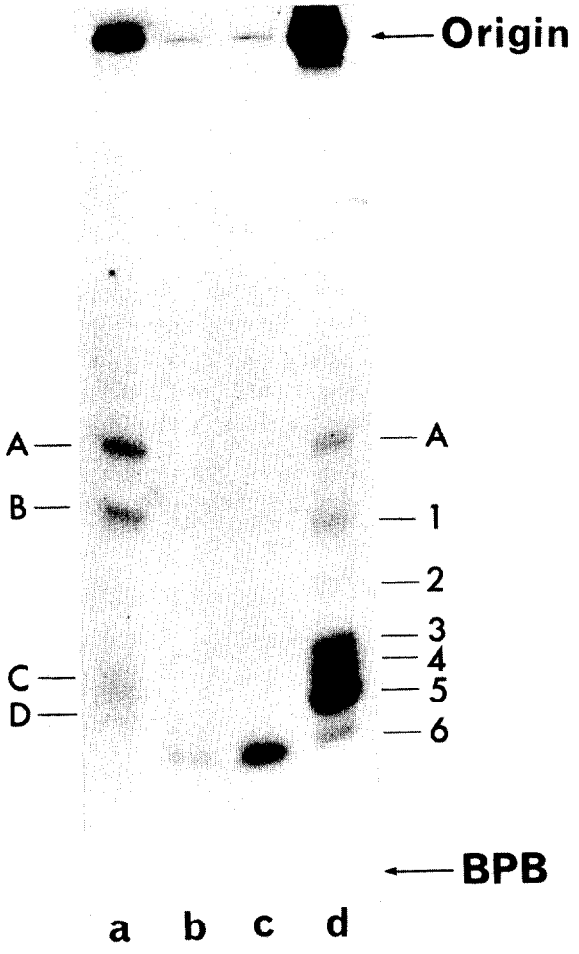

Fig. 3. Autoradiogram of 10% polyacrylamide gel electropherogram. a: T4 RNA made in TS241 at 42°C. d: T4 RNA made in 4273 at 42°C. b: The cleavage products of band D of a. c: Band 6 treated with the cell-free extract. The bands D and 6 were extracted from the gel and treated with the Q13 extract as in fig. 1.

Band 2 of fig. 1a was unaltered after incubation with the crude extract and is probably equivalent to band D reported by McClain et al. [2]. In contrast, band B was cleaved by the extract into fragments of about 20 nucleotides. However, the nature of this band is not well understood, nor have the new bands migrating slower than band A been fully characterized, because of their low yield. We know, however, that these bands disappeared when treated with the crude extract.

As seen in fig. 1a, considerable radioactivity still remained at the origin of the gel after electrophoresis. When the material was recovered and treated with the Q13 extract, no discrete band was formed on a 10% polyacrylamide gel. It seems, therefore, that there is no RNA molecule related to T4 tRNA left at the origin of the gel.

So far, attempts to identify any RNA molecule accumulating at high temperature which are related to the remaining four tRNAs (leucine, glutamine, α, and δ) in TS241 have been unsuccessful. In wild

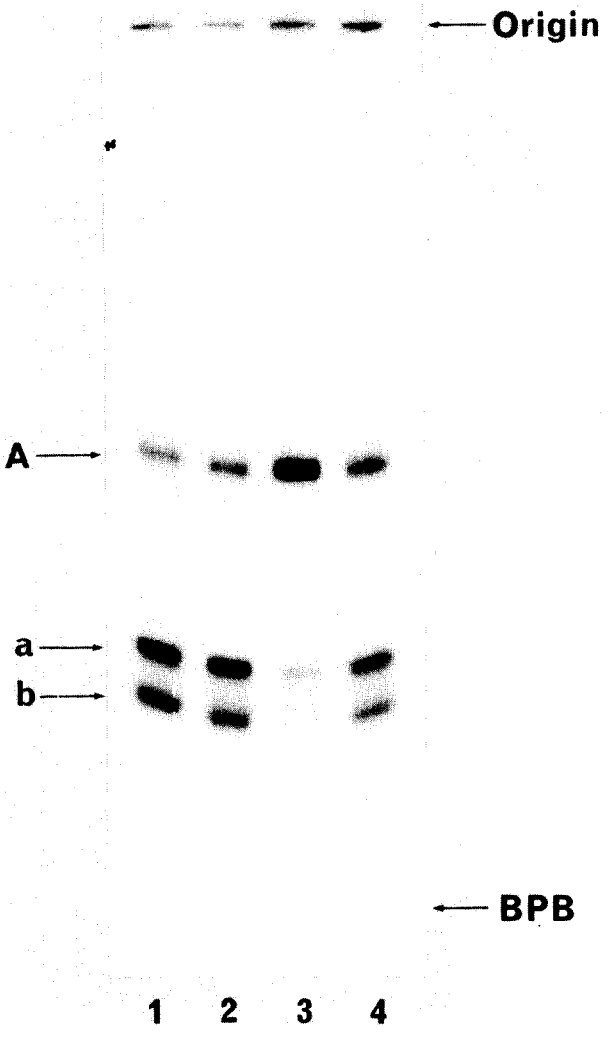

Fig. 4. Separation of the cleavage products of band A. Band A from T4 infected TS241 was incubated with S30 cell-free extracts from 4273 (1 and 2) or from TS241 (3 and 4) grown at 30°C. Incubation was performed for 15 min either at 30°C (1 and 4) or 45°C (2 and 3). Prior to incubation at 45°C, the extracts were prewarmed at 49°C for 3 min. After incubation, RNA was prepared and electrophoresed as in fig. 1.

type *E. coli* (Bb) infected with T4 phage, Guthrie et al. [9] found two other dimeric structures of T4 tRNAs; those of leucine–glutamine tRNAs and of α–δ tRNAs. In T4-infected TS241, however, none of these molecules was detectable. It is possible that the precursors for these four tRNA species are not synthesized in TS241. Alternatively, these precursors could be more labile at high temperature than those detected in the mutant.

The present results indicate that, in TS241, a dimeric precursor molecule for serine–proline tRNAs and monomeric precursors for glycine tRNA and for possibly ϵ tRNA of T4 phage are accumulated at high temperature. The exact relationships between these precursor molecules and their corresponding mature tRNAs will be elucidated when complete sequences of these precursors are clarified. In any case, it is likely that TS241 is defective in cleaving these precursor molecules at high temperature. To test this possibility, we prepared the S30 extract from TS241 grown at 30°C and incubated with band A (from fig. 1a) at two different temperatures. When incubated at 30°C (fig. 4(4)), the band was cleaved into band a (corresponding to serine tRNA) and band b (corresponding to proline tRNA). In contrast, if band A was treated with the mutant extract at high temperature, little cleavage was observed (fig. 4(3)). On the other hand, band A was cleaved at both temperatures by crude extracts prepared from wild type cells (4273) as shown in fig. 4(1) and (2). Similarly, cleavage of band D to band 6 was also shown to be temperature sensitive if the mutant extract, but not the 4273 extract, was used (data not shown). Thus, if we assume that TS241 has a single mutation, the same function must participate in cleaving both the dimeric and the monomeric precursor molecules accumulated in the mutant. It is possible that these precursor molecules have a common characteristic feature which is recognized by the same enzyme. In view of the fact that the dimeric structure is cleaved into the two tRNA constituents, the TS241 function seems to be, in all likelihood, endonucleolytic. Presumably, other nucleolytic activities are also needed for maturation of T4 tRNAs. Any defect of these enzymes by a mutation may cause the accumulation of other precursor molecules, which, in turn, should represent intermediates in the tRNA maturation process.

Acknowledgements

We thank Dr. Daniel Nathans for reading of the manuscript. This work was supported by a Scientific Research Grant from the Ministry of Education of Japan.

References

[1] Scherberg, N. H. and Weiss, S. B. (1970) Proc. Natl. Acad. Sci. U.S. 67, 1164.
[2] McClain, W. H., Guthrie, C. and Barrell, B. G. (1972) Proc. Natl. Acad. Sci. U.S. 69, 3703.
[3] Wilson, J. H., Kim, J. S. and Abelson, J. N. (1972) J. Mol. Biol. 71, 547.
[4] Nierlich, D. P., Lamfrom, H., Sarabhai, A. and Abelson, J. N. (1973) Proc. Natl. Acad. Sci. U.S. 70, 179.
[5] Sakano, H., Yamada, S., Ikemura, T., Shimura, Y. and Ozeki, H. Nucleic Acid Res. (in press).
[6] Nathans, D. (1965) J. Mol. Biol. 13, 521.
[7] Barrell, B. G. (1971) in: Proc. Nucl. Acid. Res. (Cantoni, G. L. and Davis, D. R., eds.), Vol. 2, p. 751. Harper and Row, New York.
[8] Pinkerton, T. C., Paddock, G. and Abelson, J. N. (1972) Nature New Biol. 240, 88.
[9] Guthrie, C., Seidman, J. G., Altman, S., Barrell, B. G., Smith, J. D. and McClain, W. H. (1973) Nature New Biol. 246, 6.