# REACTIONS AT THE 3' TERMINUS OF TRANSFER RIBONUCLEIC ACID. IX. tRNA NUCLEOTIDYLTRANSFERASE ACTIVITY IN BACTERIOPHAGE T4-INFECTED ESCHERICHIA COLI

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SUMMARY: There was no detectable increase in tRNA nucleotidyltransferase activity upon infection of Escherichia coli A19 with bacteriophage T4. Three mutant strains which contained low levels of tRNA nucleotidyltransferase activity also showed no increase in activity after infection. tRNA nucleotidyltransferase was purified from both uninfected and T4-infected cells and examined for possible modification. It was found that enzyme purified from both types of cells eluted from DEAE cellulose at the same specific conductivity. In addition, the molecular weight of tRNA nucleotidyltransferase purified from both uninfected and T4-infected cells was approximately 45,000 daltons as determined by chromatography on Sephadex G-100. These results suggest that T4-infection does not lead to synthesis of a new virus-specific tRNA nucleotidyltransferase nor does it cause modification of the host enzyme.

INTRODUCTION: The 3' terminus of all transfer RNA molecules contains the common trinucleotide sequence, -CCA. Enzymes (tRNA nucleotidyltransferases) which catalyze the incorporation of AMP and CMP residues into the terminal sequence of tRNA have been purified from a variety of sources (1). However, at the present time it is not known whether these terminal residues are added during transcription, or post-transcriptionally by tRNA nucleotidyltransferase. A precursor of $tRNA^{Tyr}$ has been isolated from ϕ80-infected E. coli (2) and been shown to already contain the -CCA sequence as well as three additional nucleotides at the 3' end. From these data, it was concluded that the -CCA terminus is synthesized during transcription. On the other hand, labeling of tRNA in the presence of actinomycin (3,4), hybridization studies (5) and the existence of the enzyme tRNA nucleotidyltransferase all suggested that the -CCA sequence may be added in a post-transcriptional process.

As part of a continuing study of the synthesis and function of the -CCA terminus of tRNA, we have attempted to ascertain the relationship of tRNA

nucleotidyltransferase to the biosynthetic process. Infection of E. coli with various bacteriophages of the T-series is known to induce the synthesis of virus-specific tRNAs containing the usual 3' terminal sequence (6-8). However, recent evidence indicates that the -CCA terminus is absent from the tRNA precursors isolated from T4 (J. Abelson, personal Communication), suggesting that these nucleotides are added post-transcriptionally presumably by tRNA nucleotidyltransferase. Since phage infection often leads to the production of enzymes involved in macromolecular synthesis even though the host might contain similar activities (9), it was of interest to determine whether T4 infection might also lead to the synthesis of a new tRNA nucleotidyltransferase.

In this paper the levels of tRNA nucleotidyltransferase were examined in uninfected and T4-infected E. coli A19, as well as in several mutants containing low levels of the host enzyme. In addition, the enzyme was purified from both uninfected and infected cells. The results suggest that T4 infection causes neither the synthesis of a new tRNA nucleotidyltransferase nor the modification of the host enzyme.

MATERIALS AND METHODS: The organisms used in this study were Escherichia coli A19 ($Met^-$, RNase $I^-$) (10) and three nitrosoguanidine-induced mutants of E. coli A19 (5C15, 15A16 and 15A39) which contained low levels of tRNA nucleotidyltransferase (M. Deutscher and R. Hilderman, in preparation). All experiments were performed in YT liquid media (8 g tryptone, 5 g yeast extract and 5 g NaCl per liter) or on YT plates.

T4 infection of cells was performed as follows: cultures of 20 ml, grown to a density of 3-4 x $10^8$ cells per ml, were cooled in ice, and a 2 ml sample was removed for the uninfected control. L-tryptophan was added to a final concentration of 10 µg/ml followed by infection with T4 at a multiplicity of 4-5. The infected culture was left in ice for 10 min to allow phage adsorption and then reincubated at 37 C with shaking (zero time of infection). Samples of 2 ml were removed at 2,8,15,20 and 25 min after infection, centrifuged at 2,100 x g for 5 min, and the pellets suspended in 1 ml of 0.05 M

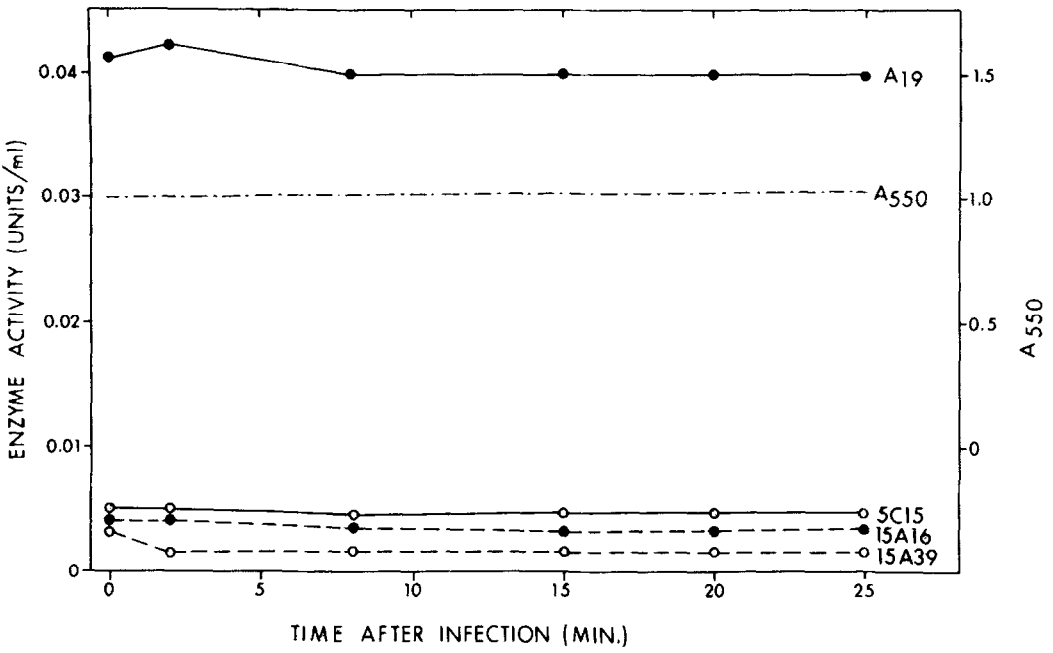


Fig. 1. tRNA Nucleotidyltransferase Activity at Various Times after T4 Infection. Cells grown to 3-4 x $10^8$ cells per ml were infected with T4 at a multiplicity of 4-5. Aliquots were removed at the indicated times and cell extracts prepared as described in Materials and Methods. Concentrated cell extracts (200 µl) were assayed in duplicate in the presence or absence of tRNA for 15 min at 37 C.

glycine buffer, pH 9.0. Cell growth was monitored throughout the experiment by the absorbance at 550 nm. The cell suspensions described above were sonicated for a total of 2 min in 1 min bursts with 30-sec cooling using a Sonifier Cell Disruptor at a setting of 1. The entire procedure was performed with the sample immersed in an ice bath.

The assay for tRNA nucleotidyltransferase in extracts measures the incorporation of $\alpha$-$^{32}$P-ATP into acid-precipitable material. Reaction mixtures contained in 0.4 ml: 50 mM glycine buffer, pH 9.0; 10 mM $MgCl_2$; 1 mM ($\alpha$-$^{32}$P) ATP (specific activity about $10^3$ cpm/nmole); and 200 µg yeast tRNA (60% tRNA-C-C). Assays were also performed in the absence of tRNA in order to evaluate extraneous incorporation, probably due to polynucleotide phosphorylase and poly(A) polymerase. These reactions amounted to less than 20% of the tRNA-dependent activity, and have been subtracted in all the data presented. Reactions were

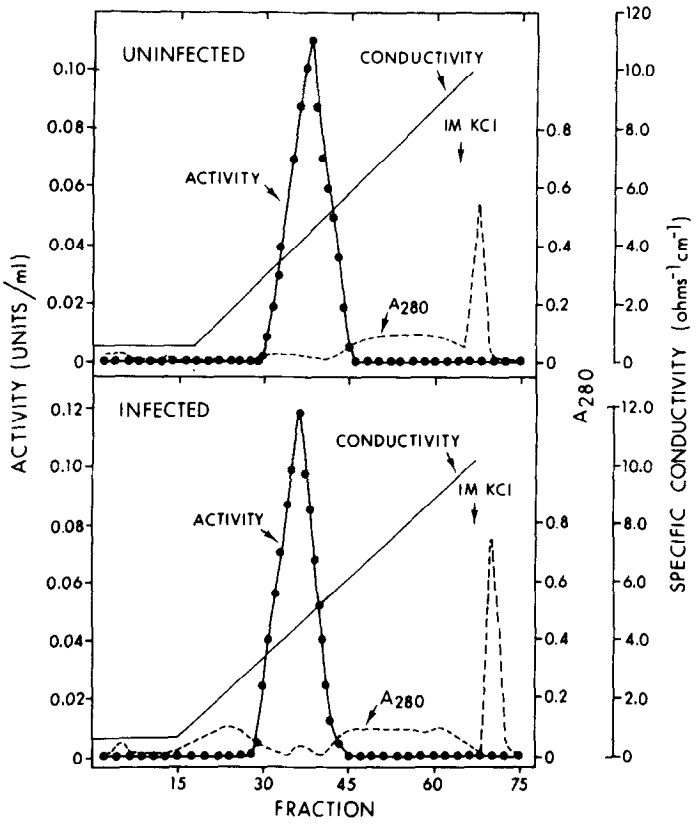


Fig. 2. DEAE-Cellulose Chromatography of tRNA Nucleotidyltransferase from Uninfected and T4-Infected Cells. Dialyzed ammonium sulfate fractions from uninfected cells (81 total units) or infected cells (91 total units) were put on a DEAE-cellulose column (1.5 by 8 cm), and washed with Buffer A. Activity was eluted with a linear gradient (200 ml) from Buffer A to the same buffer containing 0.20 M KCl. Fractions of 4 ml were collected. Aliquots of 15 µl were assayed for AMP-incorporating activity.

begun by addition of 0.2 ml of cell extract, incubated at 37 C for 5 min, and stopped with 3 ml of 10% trichloroacetic acid (TCA) containing 0.02 M sodium pyrophosphate. After 10 min in ice, the precipitate was collected on Whatman GF/C filter paper discs, washed six times with 3 ml of 2.5% TCA-0.02 M sodium pyrophosphate, and one time with ethanol-ether (1:1). Filters were dried under an infrared lamp and counted in toluene-based scintillation fluid.

tRNA nucleotidyltransferase was purified by a modification of the procedure described by Preiss et al. (11) using 3 g of wet bacterial paste from uninfected or T4-infected cells. Extracts were prepared by suspending the

bacterial paste in 3 ml of 0.05 M Tris-Cl, pH 7.4 - 0.01 M $MgCl_2$. Glass beads (8 gm, 0.5 mm) were added and this suspension was homogenized for 15 min at a setting of 2 at -5 C in a precooled Sorvall omni-mixer. Additional buffer (10 ml) was added and the suspension was homogenized 10 min more. All further procedures were carried out at 4 C. A crude extract was prepared by centrifuging the homogenate at 3,100 x g for 5 min. The crude extract was further centrifuged in a Beckman model L3-50 ultracentrifuge at 105,000 x g for 90 min to remove ribosomes. Nucleic acids were partially removed from the high speed supernatant fraction by addition of 1 ml of 5% streptomycin sulfate to each 5 ml of supernatant. This mixture was stirred for 10 min and the precipitate removed by centrifugation at 13,000 x g for 5 min. Dithiothereitol was added to the supernatant at this point to a final concentration of 0.1 mM. Ammonium sulfate (4.6 gm) was slowly added with stirring to the supernatant fluid (12.8 ml). After 15 min the mixture was centrifuged at 13,000 x g for 5 min, the pellet was resuspended in 3.2 ml of 0.01 M Tris-Cl, pH 7.4; 1.0 mM $MgCl_2$; 0.1 mM DTT; 0.1 mM EDTA and 10% glycerol (Buffer A). The solution was dialyzed against 1500 ml of Buffer A overnight.

RESULTS: The level of tRNA nucleotidyltransferase activity at various times after infection was determined in extracts prepared from E. coli A19 and from three mutants which contained less than 20% of the normal amount of enzyme (Fig. 1). The constant $A_{550}$ value throughout the period of observation indicated that all the cells had been infected; in uninfected control cultures this value increased by 0.5 units during the 25 min of incubation. The figure also shows that tRNA nucleotidyltransferase activity in A19 remained essentially constant during the 25 min of infection, suggesting that new enzyme was not being made.

Since it was possible that a virus-specific enzyme was not detected because its appearance exactly paralleled the disappearance of host enzyme, tRNA nucleotidyltransferase activity was also examined in host mutants. The low background level of activity in these strains would also allow detection of

TABLE I

PLATING EFFICIENCY OF T4 ON A19 AND ON tRNA NUCLEOTIDYLTRANSFERASE MUTANTS.

| Organism | Titer [a] | % Relative Efficiency |
|---|---|---|
| A19 | $2.74 \times 10^{11}$ | 100 |
| 5C15 | $2.40 \times 10^{11}$ | 88 |
| 15A39 | $2.33 \times 10^{11}$ | 85 |
| 15A16 | $2.35 \times 10^{11}$ | 86 |

Cells were grown to a density of 3-4 x $10^8$ cells/ml at 37 C and infected with a constant amount of phage.

[a] The phage titer is expressed as PFU/ml of phage stock.

a virus-specific enzyme made in extremely small amounts. However, as shown in Fig. 1, no evidence could be obtained for the induction of a phage enzyme even in the mutant hosts. These data indicate that if a phage activity were induced, it would be present at extremely low levels (less than 5% of that in uninfected cells).

In order to determine whether T4 could undergo a normal infectious cycle in A19 and the mutant strains, the plating efficiency of the phage on these various cells was determined. The data in Table I indicate that all of the cells were equally efficient for plating T4. In addition, a one-step growth curve with E. coli A19 gave a burst size of about 60 phage, although lower phage yields were observed with the mutants (unpublished observations). These results suggest that the low activity in the mutants after T4 infection is probably not due to the inability of the phage to reproduce in these cells.

tRNA nucleotidyltransferase activity has a pH optimum which is quite alkaline. Thus, the possibility existed that we did not detect a phage-induced enzyme because of incorrect assay conditions. In order to eliminate this possibility, tRNA nucleotidyltransferase was assayed in extracts of uninfected and infected cells at a variety of pH values. Activity from both types of cells had identical pH profiles with optima between pH 9 and 10. Similarly, the response of tRNA nucleotidyltransferase to varying concentrations of ATP, $Mg^{++}$ and tRNA was identical in extracts from normal and infected cells.

In the case of several enzymes involved in macromolecular synthesis, T4 infection leads to a modification of the host protein rather than synthesis of a totally new enzyme (12,13). In order to investigate this possibility, tRNA nucleotidyltransferase was purified from both uninfected and infected cells. The purification scheme for both enzymes is presented in Tables 2A and 2B. Each enzyme was purified approximately 180-fold from the crude extract. The specific activity and yield of the two enzymes were essentially identical at every step of purification.

The elution profile on DEAE cellulose of each of the enzymes is shown in Fig. 2. Each enzyme eluted as a single peak, and in essentially the same position. No further activity was recovered even with a wash of 1 M KCl. The specific conductivity at the peak was 4.36 $ohm^{-1}$ $cm^{-1}$ for the uninfected cell enzyme and 4.45 $ohm^{-1}$ $cm^{-1}$ for the enzyme from infected cells. The elution profile of both purified enzymes on Sephadex G-100 is presented in Fig. 3. Each enzyme eluted in the same position which corresponded to a molecular weight of 45,000 daltons. This molecular weight is in agreement with that of other workers (14,15). These data indicate that the tRNA nucleotidyltransferase from T4-infected cells has the same charge and molecular weight as the enzyme from uninfected cells, and suggest that the protein has not been modified upon infection.

DISCUSSION: The data presented in this paper provide evidence that infection of *E. coli* with T4 does not lead to the induction of a new tRNA nucleotidyl-

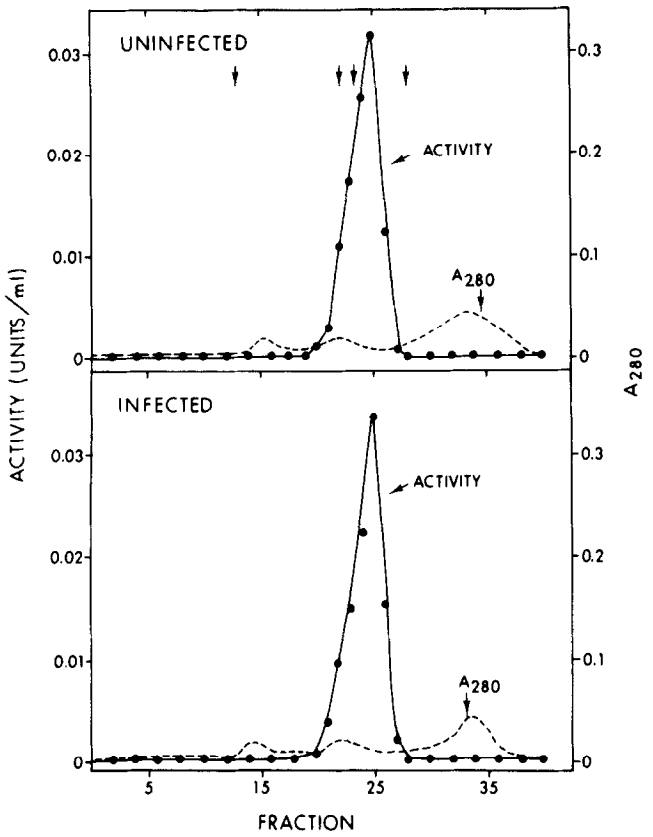


Fig. 3. Sephadex G-100 Chromatography of tRNA Nucleotidyltransferase from Uninfected and T4-Infected Cells. The combined peak fractions from DEAE-cellulose uninfected cells (29 units) and infected cells (32 units) were concentrated to 2 ml by ammonium sulfate precipitation and placed on a column (0.9 by 50 cm) of Sephadex G-100. The column was eluted with Buffer A. Fractions of 1 ml were collected. Aliquots of 100 µl were assayed for AMP-incorporating activity. Arrows from left to right represent the elution position of the standard proteins aldolase (160,000), alkaline phosphatase (80,000), bovine serum albumin (68,000) and chymotrypsinogen (25,000).

transferase or to modification of the host enzyme. The use of host mutants containing low levels of tRNA nucleotidyltransferase would have allowed detection of extremely small amounts of a new activity, but none was found. We also think it unlikely that a phage-specific enzyme was lost during purification of the uninfected and infected cell proteins since essentially identical yields and specific activities were obtained at each step. These results suggest that if tRNA nucleotidyltransferase is involved in synthesis of the -CCA terminus of tRNA, T4-specific tRNA molecules utilize the host enzyme for

TABLE 2A

PURIFICATION OF tRNA NUCLEOTIDYLTRANSFERASE FROM UNINFECTED E. COLI A19

| | Fraction | Total Units | Specific Activity [a] | % Yield [b] | Relative Purification |
|---|---|---|---|---|---|
| 1. | Crude extract | 10.8 | 0.009 | 100 | -- |
| 2. | 105,000 x g supernatant | 11.0 | 0.021 | 102 | 2.3 |
| 3. | Streptomycin sulfate precipitation [c] | 9.4 | 0.013 | 87 | 1.4 |
| 4. | Ammonium sulfate precipitation | 5.1 | 0.07 | 47 | 7.8 |
| 5. | DEAE-cellulose | 1.7 | 0.97 | 16 | 108 |
| 6. | Sephadex G-100 | 0.48 | 1.18 | 4 | 131 |
| 6a. | Sephadex peak | 0.03 | 1.60 | 0.3 | 178 |

[a] Specific activity was calculated on the basis of protein determination by the Lowry method (10) through fraction 4, and by $A_{280}$ measurements for fractions 5, 6, and 6a.

[b] The apparent low yields after DEAE cellulose and Sephadex G-100 chromatography were due to use of only a fraction of the recovered activity for the subsequent step. Actual recovery on DEAE cellulose was 65-70%, and on Sephadex was 40-45% for each enzyme.

[c] The lower specific activity in this step is probably due to streptomycin sulfate interfering with the protein determination.

their synthesis. This agrees with the data of Scherberg et al. (16) that T4 infection in the presence of chloramphenicol still leads to the synthesis of phage-specific tRNAs that are capable of being charged with amino acids.

The role of T4-induced tRNAs in the infectious process of this phage is still not clear since phage mutants have been isolated in which the genes

TABLE 2B

PURIFICATION OF tRNA NUCLEOTIDYLTRANSFERASE FROM T4-INFECTED E. COLI A19

| | Fraction | Total Units | Specific Activity [a] | % Yield [b] | Relative Purification |
|---|---|---|---|---|---|
| 1. | Crude extract | 11.7 | 0.009 | 100 | -- |
| 2. | 105,000 x g supernatant | 11.6 | 0.025 | 99 | 2.8 |
| 3. | Streptomycin sulfate precipitation [c] | 10.0 | 0.015 | 86 | 1.7 |
| 4. | Ammonium sulfate precipitation | 5.6 | 0.07 | 48 | 7.8 |
| 5. | DEAE-cellulose | 1.9 | 0.96 | 16 | 107 |
| 6. | Sephadex G-100 | 0.51 | 1.12 | 4 | 124 |
| 6a. | Sephadex peak | 0.03 | 1.65 | 0.3 | 183 |

specifying the tRNAs are deleted (17). Thus, it was not possible to determine directly the relationship of host tRNA nucleotidyltransferase to T4 tRNA synthesis, despite the fact that host mutants with low levels of enzyme have been isolated. However, preliminary results do suggest that host tRNA nucleotidyltransferase may play a role in T4 phage infection since of five E. coli mutants examined all gave extremely small phage yields (< 20% of wild-type) in one-step growth experiments (R. Hilderman and M. Deutscher, unpublished results). In addition, the finding that the -CCA sequence is absent from T4 tRNA precursors also suggests that host tRNA nucleotidyltransferase is involved in T4 tRNA biosynthesis.

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